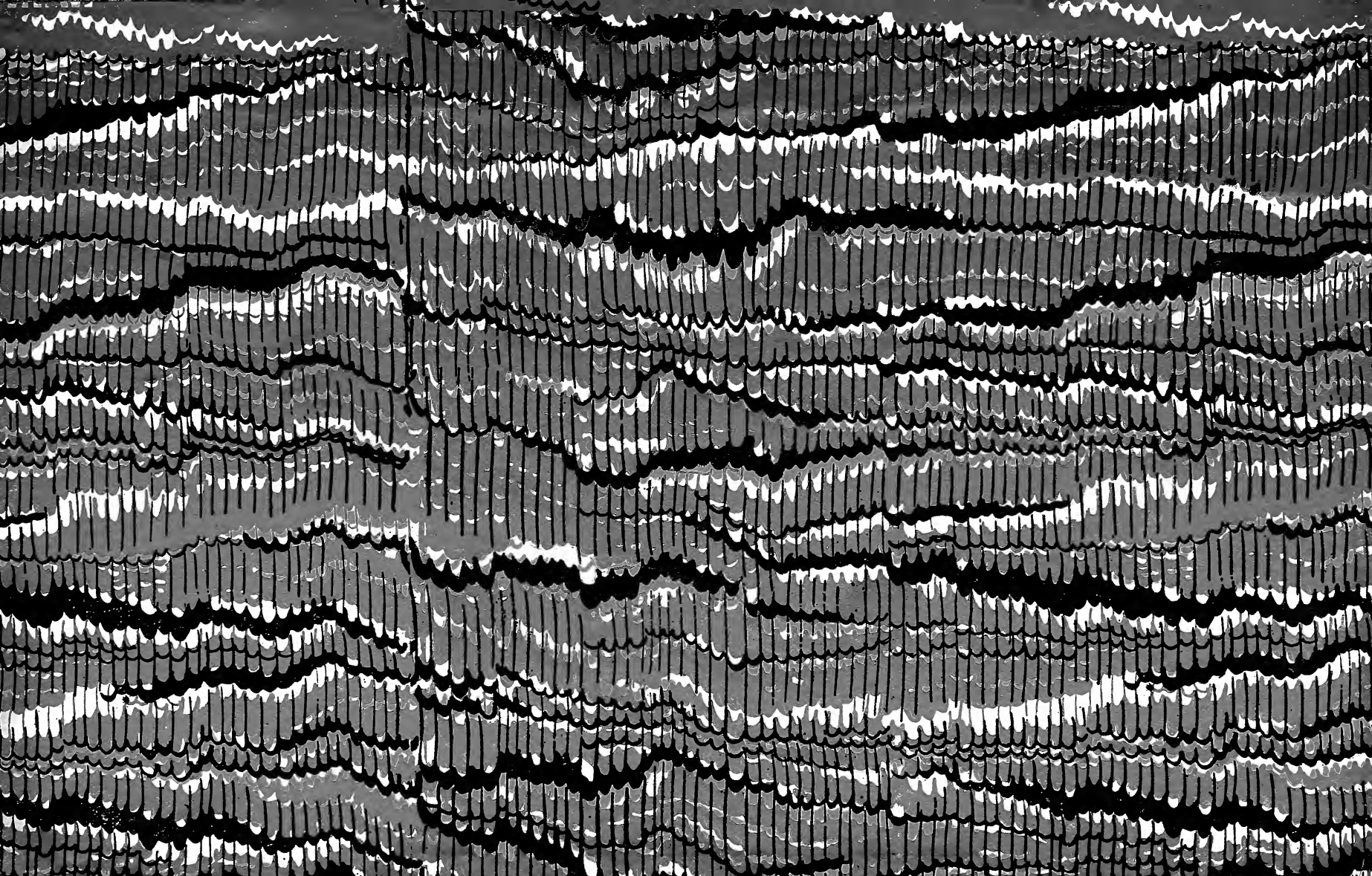

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BY THE

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ERRATA, VOLUME X

Page 47, line 13. For *Tristanea*, read *Tristania*.
Page 73, line 11. For *Mangus*, read *Magnus*.
Page 85, line 38. For *temate*, read *tomato*.
Page 109, line 41. For *pentendra*, read *pentandra*.
Page 144, line 12. For *minimum*, read *maximum*.
Page 181, line 23. For *Thysonotis*, read *Thysanotus*.
Page 185, line 19. For *Myrtiaceae*, read *Myrtaceae*.
Page 248, line 10. For *Juniperi-virginiani*, read *Juniperi-virginianae*.
Page 358, 11th line from the bottom. To *R. Hertwig* **I.**, add *215–232*.
Page 393, line 8. For *in well*, read *as well*.
Page 395, last line. For *affected*, read *effected*.
Page 415, line 7. For *B. dysenteriae*, read *Bacillus dysenteriae*.
Page 438, footnote, line 2. For *Grund Beeinflussung*, read *Grund der Beeinflussung*. For *H-ion*, read *H-Ionen*.
Page 487, line 17. For *Astragalinae*, read *Astragalanae*.

Vol. X JANUARY, 1923 No. 1

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AMERICAN
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VOL. X JANUARY, 1923 NO. 1

REVISION OF THE SOUTH AMERICAN SPECIES OF CUSCUTA

II

T. G. YUNCKER

(Received for publication March 25, 1922)

Section CLISTOGRAMMICA Engelmann

KEY TO THE SUBSECTIONS

Flowers not subtended by bracts; calyx segments more or less united.....PLATYCARPAE.
Flowers subtended by numerous bracts; calyx segments mostly free (no members of this subsection have been found from South America).....LEPIDANCHE.

Subsection PLATYCARPAE Engelmann

KEY TO THE SPECIES

Calyx lobes obtuse (acutish in *C. gymnocarpa*, *C. suaveolens*, and *C. parviflora*).
Calyx lobes mostly not overlapping, or but slightly so, mostly smooth.
Styles becoming subulate and divaricate in fruit.....25. *C. obtusiflora.*
Styles not becoming noticeably subulate in fruit (subulate in *C. pentagona subulata*).
Flowers mostly more than 2 mm. long.
Scales prominent, withered corolla about the capsule or at its base.
Corolla lobes acute.
Calyx nearly as long as the corolla, lobes ovate, may be slightly overlapping, corolla lobes upright or reflexed.
Corolla lobes spreading or reflexed, tips inflexed. 26. *C. pentagona.*
Corolla lobes upright.....27. *C. gymnocarpa.*
Calyx much shorter than the corolla, lobes triangular, not at all overlapping, corolla lobes upright, tips inflexed.....28. *C. suaveolens.*
Corolla lobes mostly obtuse.
Calyx lobes not overlapping.....29. *C. racemosa.*
Calyx lobes more or less overlapping.....30. *C. platyloba.*

[The **Journal** for December (**9**: 535–581) was issued Jan. 10, 1923.]

Scales narrow, not prominent, withered corolla capping the capsule.....31. *C. stenolepis.*
Flowers mostly less than 2 mm. long.....32. *C. parviflora.*
Calyx lobes broadly overlapping, frequently keeled.....33. *C. cristata.*
Calyx lobes acute.
Flowers fleshy, corolla lobes more or less papillate, tips mostly inflexed.....34. *C. indecora.*
Flowers not fleshy, corolla lobes not inflexed at the tips.
Infrastamineal scales present.
Pedicels mostly longer than the flowers, calyx lobes triangular. 35. *C. globosa.*
Pedicels no longer than the flowers (mostly shorter), calyx lobes ovate.
Corolla lobes mostly shorter than the tube.....36. *C. micrantha.*
Corolla lobes mostly equal to or longer than the tube.....37. *C. acuta.*
Infrastamineal scales lacking.....38. *C. insquamata.*

25. *Cuscuta obtusiflora* Humboldt, Bonpland, & Kunth (Pl. V, fig. 29, *A–E*)

Cuscuta obtusiflora Humboldt, Bonpland, & Kunth, Nova gen. et spec. pl. 3: 122 (96 in folio edition). 1818.—Engelmann, Trans. Acad. Sci. St. Louis 1: 492. 1859.—Progel in Martius, Flora Brasiliensis 7: 380, Pl. 127, fig. 1. 1871.
Cuscuta inodora Willdenow, in herb., ex Engelmann, Trans. Acad. Sci. St. Louis 1: 492. 1859; in synon.

Stems medium. Flowers 2–2.5 mm. long, subsessile in dense glomerulate clusters, more or less glandulous; calyx scarcely as long as the corolla; lobes unequal, ovate, obtuse, not overlapping; corolla campanulate; lobes about equal to the tube, ovate, obtuse or more rarely acutish, upright, becoming reflexed in fruit; stamens shorter than or nearly equaling the lobes and commonly placed directly in the sinuses, stout, subulate filaments longer than the oval anthers; scales spatulate, fringed about the top, mostly not reaching the stamens, bridged below the middle; styles about as long as the globose ovary, becoming subulate. Capsule depressed-globose, exposed, with the withered corolla about it towards the base, intrastylar aperture large, styles becoming subulate and divergent; seeds globose, compressed, slightly rostrate, 1.25–1.5 mm. long; hilum linear, perpendicular.

All the specimens from South America belong to Engelmann's variety *vera*. The above description was drawn from Humboldt's type specimen.

Type locality: In the Peruvian Andes. *Distribution:* Colombia, Brazil, and southward to Peru and in Paraguay and Uruguay.

Specimens examined: Brazil: Matto Grosso, Corumba (Hoehne *4016*).
Colombia: (Triana, without date or number); Medellin (Triana *2178*, and in 1851–57).
Paraguay: River Ypacarai (Hassler *3918*).
Peru: (Humboldt, the type, a fragment in the Engelmann herbarium).
Uruguay: (Lorentz *64*).

26. *Cuscuta pentagona* Engelmann

For the synonymy, description, and illustration of this species see Yuncker, Ill. Biol. Monogr. **6**: 140. 1921.

KEY TO THE SOUTH AMERICAN VARIETIES

Styles slender, not becoming subulate in fruit.
Calyx lobes overlapping, forming angles at the sinuses; flowers relatively small. *typica.*
Calyx lobes not at all, or but slightly, overlapping and not forming angles at the sinuses; flowers usually larger..... *calycina.*
Styles becoming subulate in fruit, intrastylar opening large..... *subulata.*

Cuscuta pentagona typica

Specimens examined: ARGENTINA: Buenos Aires (Venturi *100*).
URUGUAY: Montevideo (Fruchard in 1871, and in 1875; Courbon *146*).

Cuscuta pentagona calycina Engelmann

Specimen examined: BRAZIL: Itajahy (Ule *487*).

Cuscuta pentagona subulata n. var.

Styles becoming subulate, capsule and intrastylar aperture large, scales scarcely reaching, or reaching, the stamens.

Type locality: Maraham, Brazil. *Distribution:* Brazil and Ecuador.

Specimens examined: BRAZIL: (Glaziou *19677* in part); Maraham (Gardner *6068* in part, the type, a fragment in the Engelmann herbarium); Prov. São Paulo (St. Hilaire *C*[1] *1271*); Prov. Santa Catharina (Pabst *565*).
ECUADOR: Guayaquil (Jameson *542*).

27. *Cuscuta gymnocarpa* Engelmann
(Pl. IV, fig. 22, *A–E*)

Cuscuta gymnocarpa Engelmann, Trans. Acad. Sci. St. Louis 1: 496. 1859.

Stems slender to medium. Flowers about 2 mm. long, becoming 2.5–3 mm. long in fruit with the enlarged capsule, on pedicels about as long as the flowers, in few-flowered, globose, umbellate clusters; calyx slightly shorter than the corolla tube, lobes ovate, obtuse or slightly acutish, not overlapping; corolla campanulate, thin, lobes triangular, acute, upright, with tips frequently inflexed, shorter than the tube; scales reaching the stamens, ovate, fringed with moderate processes, bridged below the middle; stamens shorter than the lobes, anthers oval, about equal to the stout subulate filaments; styles about equal to, or shorter than, the globose ovary. Capsule globose or slightly depressed-globose, the withered corolla remaining at the base, not circumscissile. Seeds two to four in each capsule, about 1.5 mm. long, oval, hilum prominent, oblique or perpendicular.

This species differs from *C. pentagona* in having shorter, upright corolla lobes, shorter filaments, and more globose capsules. It differs from *C. acuta* in having the corolla at the base of the protruding capsule, shorter corolla lobes and styles, and obtuse calyx lobes.

Type locality: "James Island of the Galapagos Group." *Distribution:* Galapagos Islands.

Specimens examined: GALAPAGOS ISLANDS: Albemarle Island, Tagus Cove (Stewart *3092*), Cowley Bay (Bauer *205*).

28. *Cuscuta suaveolens* Seringe

Cuscuta suaveolens Seringe, Ann. Sci. Phys. Nat. Lyon **3**: 519. 1840.—Gay, Hist. de Chile **4**: 448. 1849.
Cuscuta corymbosa Choisy, Mém. Soc. Phys. Hist. Nat. Genève **9**: 276. 1841; and in De Candolle, Prodromus **9**: 456. 1845. Not Ruiz & Pavon.
Cuscuta Hassiaca Pfeiffer, Bot. Zeit. **1**: 705. 1843.
Engelmannia migrans Pfeiffer, Bot. Zeit. **3**: 674. 1845.
Engelmannia suaveolens Pfeiffer, Bot. Zeit. **4**: 21. 1846.
Cuscuta diaphana Wenderoth, Fl. Hassiaca, p. 346. 1846.
Pfeifferia suaveolens Buchinger, Ann. Sci. Nat. Bot. III, **5**: 88. 1846.
Cassutha suaveolens Des Moulins, Études organiques sur les Cuscutes, p. 66. 1853.
Cuscuta popayanensis Poeppig in herb., ex Engelmann, Trans. Acad. Sci. St. Louis **1**: 505. 1859. Not H. B. K.
Cuscuta chilensis Bertero in sched., ex Engelmann, Trans. Acad. Sci. St. Louis **1**: 505. 1859. Not Ker-Gawler.
Cuscuta racemosa chiliana Engelmann, Trans. Acad. Sci. St. Louis **1**: 505. 1859.—Yuncker, Ill. Biol. Monogr. **6**: 144, figs. 36 and 94. 1921.
? Cuscuta floribunda Philippi, Fl. Atac., p. 37. 1860. Not H. B. K.
? Cuscuta andina Philippi, Anal. Univ. Chile **90**: 225. 1895.
? Cuscuta racemosa floribunda Reiche, Anal. Univ. Chile **120**: 819. 1907.
? Cuscuta racemosa andina Reiche, Anal. Univ. Chile **120**: 819. 1907.

Stems slender to medium. Flowers 3–4 mm. long, more or less glandular, membranous, on pedicels mostly shorter than the flowers, in racemose clusters; calyx lobes shorter than the corolla tube, triangular-ovate, acutish, not overlapping, sinuses more or less rounded, edges sometimes revolute; corolla campanulate or funnel-form, becoming globular about the developing capsule; lobes ovate-triangular, upright, with acute, inflexed tips, somewhat shorter than the tube; anthers shorter than the lobes, filaments subulate, about equal to the oval anthers; scales not reaching the stamens (infrequently barely reaching them), oblong-ovate or triangular, fringed with medium processes, bridged below the middle; ovary globose, styles slender, about equal to the ovary. Capsule globose, with the withered corolla about it, not circumscissile, two- to four-seeded, seeds 1.5–2 mm. long, roundish, hilum oblong, perpendicular.

Most of the writers have followed the example of Engelmann in considering this a variety of *C. racemosa* Mart. It is undoubtedly closely related to that species, but it is believed that it exhibits characters that warrant its being segregated as a separate species. It is easily distinguished by its large flowers which are light-colored, and also by the rounded sinuses of the calyx.

Type locality: Lyons, France, where it grew from seeds imported from Chile. *Distribution:* Chile, Paraguay, and Uruguay.

Specimens examined: CHILE: (Bertero *201* and *940;* Poeppig, probably the type of *C. popayanensis* [not H. B. K.]); St. Jago (Gay *449*); Rancagua (Bertero *205*, taken to

represent the type of Choisy's *C. corymbosa*); St. Augustin de Tango (Reed in 1867); Valparaiso (Rusby *2000*); Valdivia (Lechler *479*); Prov. Santiago (Philippi in 1861 and in 1862).

PARAGUAY: Montevideo (Fruchard).

URUGUAY: Salto (Osten *3324*).

29. *Cuscuta racemosa* Martius

Cuscuta racemosa Martius in Spix and Martius, Reise Bras. 1: 286. 1823.—Choisy, Mém. Soc. Phys. Hist. Nat. Genève 9: 277, Pl. 3, fig. 1. 1841; and in De Candolle, Prodromus 9: 456. 1845.

Cuscuta racemosa brasiliana Engelmann, Trans. Acad. Sci. St. Louis 1: 505. 1859.—Progel in Martius, Flora Brasiliensis 7: 384, Pl. 125. 1871.

Stems slender, smooth or rarely papillate. Flowers 2.5–4 mm. long, more or less glandular, on pedicels mostly about as long as the flowers, in loose, racemose clusters, membranous or somewhat fleshy, straw-colored to deep red; calyx shorter than the tube, or equaling it in one variety; lobes ovate, obtuse or rarely somewhat acutish; corolla campanulate, lobes ovate, obtuse or rarely slightly acutish, tips inflexed or straight; stamens shorter than the lobes, the oval anthers about equal to the stoutish, subulate filaments; scales mostly about reaching the stamens, oblong, copiously fringed particularly about the upper half, bridged below the middle; stigmas ordinarily large and frequently flattened, styles stoutish and not infrequently more or less subulate, equal to, or longer than, the globose or obovate ovary which is thickened at the top. Capsule globose, carrying the withered corolla about it or toward its base; seeds one to four in each capsule, oval, about 2 mm. long, hilum short, perpendicular or oblique.

KEY TO THE VARIETIES

Calyx lobes ordinarily not overlapping, longer than broad, mostly reaching beyond the middle of the corolla tube.
Capsule covered or surrounded by the withered corolla, not becoming markedly exserted..... *typica.*
Capsule becoming exserted and not covered by the withered corolla..... *nuda.*
Calyx lobes commonly more or less overlapping, about as broad as long, ordinarily not reaching the middle of the tube and frequently papillate..... *miniata.*

Cuscuta racemosa typica

(Pl. IV, fig. 23, *A–E*)

Flowers about 3 mm. long; calyx shorter than the corolla tube, lobes ordinarily not overlapping, longer than broad; corolla lobes shorter than the tube, obtuse, tips commonly inflexed.

Type locality: Province Rio de Janeiro, Brazil. *Distribution:* Central and southern Brazil.

Specimens examined: BRAZIL: (Riedel; Booz; Ule *321;* Pohl *5100*); Prov. Rio de Janeiro (Graham; Riedel *695;* Martius in 1817, taken to represent the type, a fragment in the Engelmann herbarium; Rudio; Capt. Wilkes; Burchell *2739;* Gardner *5555;* Martius *941;* Gaudichaud *507*); Petropolis (Ball in 1882); Prov. Parana (Dusén *4006, 7987, 10005, 11349*); Prov. Minas Geraes (Lindberg *167;* Hillosen *4484;* Gaudichaud *52;* St. Hilaire *D. 557;* Weddell); Caldas (Hoehne *2774*); Prov. São Paulo (Brade *6026;* Glaziou *19677* in part; Gaudichaud *345*); Mboi (Gehrt *3351*); Prov. Santa Catharina (Ule *1848*);

Ilha do Governodor, Rio de Janeiro Bay (Miers *3430*); Isl. Paqueta, Rio de Janeiro Bay (Rose and Russell *20287*); Tropical Brazil (Burchell *667A*).

Cuscuta racemosa nuda Engelmann

Cuscuta racemosa nuda Engelmann, Trans. Acad. Sci. St. Louis **1**: 505. 1859.—Progel in Martius, Flora Brasiliensis **7**: 384, Pl. 128, fig. 3. 1871.
? Cuscuta citricola Schlechtendal, Linnaea **22**: 808. 1849.
Cuscuta racemosa Regnelliana Progel in Martius, Flora Brasiliensis **7**: 384. 1871.

Calyx lobes ovate, not overlapping, nearly as long as the corolla tube; corolla lobes reflexed and about equal to the tube. Capsule depressed-globose, exserted, intrastylar aperture large; scales oblong. This variety when not in fruit looks very much like variety *brasiliana*.

Type locality: "Near Rio, Brazil." *Distribution:* Southern Brazil.

Specimens examined: Brazil: (Sellow, taken to represent the type, a fragment in the Engelmann herbarium); Prov. Minas Geraes (Regnell *III 308;* two collections of this number were examined, one collected Dec. 26, 1864, and the other Feb. 7, 1866. This is the type number of variety *Regnelliana;* Widgren in 1845).

Cuscuta racemosa miniata Engelmann

(Pl. IV, fig. 23, *F*)

Cuscuta racemosa miniata Engelmann, Trans. Acad. Sci. St. Louis **1**: 505. 1859.—Progel in Martius, Flora Brasiliensis **7**: 384. 1871.
Cuscuta miniata Martius in Spix and Martius, Reise Bras. **1**: 286. 1823.
Cuscuta racemosa minuta Choisy, Mém. Soc. Phys. Hist. Nat. Genève **9**: 277. 1841; and in De Candolle, Prodromus **9**: 456. 1845.
Cuscuta tenuicaulis Glaziou, Mém. Soc. Bot. France **3**: 491. 1911.

Stems smooth or frequently papillate, the papillations extending part way on to the calyx in some specimens; calyx very short, the lobes commonly broader than long, overlapping; flowers fleshier in texture than those of the other varieties, and reddish.

Type locality: Brazil. *Distribution:* Central and southern Brazil.

Specimens examined: Brazil: (Martius *1292*, taken to represent the type, a fragment in the Engelmann herbarium; Ackermann in 1832); Prov. Minas Geraes (Vauthier *252;* Schwacke *8208;* Glaziou *19676*, the type number of *C. tenuicaulis;* St. Hilaire B^1 *2085;* Langsdorff); Prov. São Paulo (St. Hilaire C^2 *1488;* Prov. Goyaz (Glaziou *20422*); Prov. Matto Grosso, Cuyabá (Riedel *846* in part, mixed with *C. partita*).

30. *Cuscuta platyloba* Progel

(Pl. V, fig. 27, *A–E*)

Cuscuta platyloba Progel in Martius, Flora Brasiliensis **7**: 381, Pl. 127, fig. 3. 1871.
Cuscuta racemosa calycina Engelmann, Trans. Acad. Sci. St. Louis **1**: 505. 1859.

Stems slender. Flowers 2–4 mm. long, on pedicels shorter than the flowers, in few- to many-flowered paniculate or racemose cymes; calyx about equal to the corolla tube; lobes ovate, obtuse, overlapping, entire or irregular, sometimes slightly carinate; corolla campanulate, lobes ovate, obtuse, overlapping, about equal to the tube, upright or becoming reflexed; stamens shorter than the lobes, anthers oval, about equal to, or shorter

than, the subulate filaments; scales reaching the stamens, ovate, fringed with moderate processes, bridged at about the middle or somewhat below; ovary globose, styles slender and shorter than, or becoming longer than, the ovary, stigmas medium or infrequently large and convoluted. Capsule globose, with the withered corolla about it; styles becoming stoutish and sometimes slightly subulate, not circumscissile, thin towards the base; seeds two to four in each capsule, about 1.5 mm. long; hilum oblong, oblique.

It is believed that what Progel described as *C. platyloba* is a smaller form of Engelmann's *C. racemosa calycina*. The specimens here considered closely approach *C. racemosa*, but it is believed that they show characters that will allow of their being maintained as a distinct species.

Type locality: "Montevideo." *Distribution:* Southern Brazil and Uruguay.

Specimens examined: BRAZIL: Prov. Goyaz (Glaziou *21808*); Prov. Rio de Janeiro (Ule *3565;* Glaziou *11279;* Hillosen *2538*); Prov. Santa Catharina (Pabst *89*); Central Brazil (Weddell *2124*, probably the type of *C. racemosa calycina*); southern Brazil (Sellow); Minas, Serra da Piedade (Hoehne *6187*).

URUGUAY: Montevideo (Sellow *30*, taken to represent the type, in the U. S. National Herbarium; Fruchard in 1874 and in 1875).

31. *Cuscuta stenolepis* Engelmann

(Pl. V, fig. 31, *A–E*)

Cuscuta stenolepis Engelmann, Trans. Acad. Sci. St. Louis 1: 503. 1859.

Stems slender, densely matted. Flowers few and scattering, about 2.5 mm. long, on pedicels as long as, or shorter than, the yellowish or infrequently reddish, glandular flowers, in scattered, few-flowered, paniculate or cymose clusters; calyx deeply divided, reaching the middle of the corolla tube or nearly to the sinuses in some flowers, lobes ovate, obtuse; corolla subcylindrical, lobes about half as long as the tube, oblong-ovate, obtuse, reflexed, with inflexed tips; stamens shorter than the lobes, filaments equal to, or shorter than, the oval anthers; scales about reaching the stamens, bridged at a third or a quarter of their height, narrow, sparingly fringed with a few scattered processes; styles slender, about equal to the globose, apiculate ovary. Capsule globose or conic, with the withered corolla about the apex like a hood; seeds subglobose, about 1.5 mm. long.

Type locality: Andes of Quito, Ecuador. *Distribution:* Known only from Ecuador.

Specimens examined: ECUADOR: Quito (Hall, the type, a fragment in the Engelmann herbarium; Couthouy; E. W. D. and Mary M. Holway Aug. 21, 1920); Otavalo (Holmgren *908*).

32. *Cuscuta parviflora* Engelmann

Cuscuta parviflora Engelmann, Trans. Acad. Sci. St. Louis 1: 506. 1859.—Progel in Martius, Flora Brasiliensis 7: 385, Pl. 128, fig. 5. 1871. Not Nuttall nor Willdenow.
Cuscuta micrantha Martius in herb., ex Engelmann, Trans. Acad. Sci. St. Louis 1: 506. 1859. Not Choisy.

Stems slender, matted. Flowers 1–2 mm. long, on pedicels mostly much

longer than the flowers, in loose, few-flowered, cymose clusters; calyx shorter than, or about equaling, the corolla tube; lobes ovate to triangular-ovate, obtuse or slightly acutish, slightly, if at all, overlapping; corolla widely campanulate, lobes longer than the tube, spreading or upright with inflexed tips, ovate or lanceolate, obtuse to acute; stamens shorter than the lobes or rarely longer than the lobes; oval anthers about equal to, or shorter than, the slender filaments; scales reaching the stamens, ovate, fimbriate, bridged below the middle; styles about equal to the globose ovary; capsules depressed-globose, much larger than the flower, exserted, withered corolla persistent at the base, intrastylar aperture large, not circumscissile; seeds 1–1.25 mm. long, globose, hilum oblong, perpendicular.

This is the smallest of the species seen from South America and seems to produce capsules sparingly. Only two or three mature fruits were seen in the several specimens examined.

Cuscuta parviflora typica

(Pl. V, fig. 25, *A–E*)

Flowers 1.5–2 mm. long. Corolla lobes ovate, obtuse or only slightly acutish, stamens shorter than the corolla lobes.

Type locality: Villa Rica, Brazil. *Distribution:* Southern Brazil.

Specimens examined: BRAZIL: (St. Hilaire); Villa Rica (Pohl *5726*, taken to represent the type of this, a fragment in the Engelmann herbarium); Prov. Minas Geraes, Ouro Preto (Schwacke *7560;* Ule *2652;* E. W. D. and Mary M. Holway *1374*).

Cuscuta parviflora elongata Engelmann

(Pl. V, fig. 25, *F*)

Cuscuta parviflora elongata Engelmann, Trans. Acad. Sci. St. Louis 1: 506. 1859.—Progel in Martius, Flora Brasiliensis 7: 386. 1871.

Flowers 1–1.5 mm. long. Calyx lobes acutish; corolla lobes triangular or lanceolate, acute, stamens equal to or exceeding the corolla lobes.

Type locality: Minas Geraes, Brazil (?). *Distribution:* Central and southern Brazil.

Specimens examined: BRAZIL: Goyaz (Weddell *2125*); São Paulo (St. Hilaire *C*[1] *665*).

33. *Cuscuta cristata* Engelmann

(Pl. V, fig. 28, *A–E*)

Cuscuta cristata Engelmann, Trans. Acad. Sci. St. Louis 1: 507. 1859.

Stems medium. Flowers 2.5–3 mm. long, slightly fleshy and glandular, subsessile on short, stout pedicels in few- to many-flowered, lateral, cymose clusters, perianth segments frequently uneven; calyx as long as the corolla tube; lobes broadly ovate, obtuse, frequently with an uneven, cristate carina which may extend down on to the pedicel, overlapping; corolla widely campanulate, early becoming somewhat globular about the developing capsule; lobes slightly shorter than the tube, ovate, obtuse, spreading, becoming reflexed in fruit; stamens shorter than the lobes, the oval anthers

about equal to the stout, subulate filaments; scales reaching the stamens or shorter, bridged below the middle, slightly spatulate, sparingly fringed with medium-length processes, particularly about the upper half; styles stoutish, much shorter than the large, globose ovary which is thickened at the top, stigmas very small. Capsule depressed-globose, thin towards the base where it may break away when pulled, carrying the withered corolla about it or toward the base, styles becoming divaricate, intrastylar aperture large and rhomboid; seeds about 1.5 mm. long, oblique, rostrate.

This species is distinguished mainly by the shape of its capsule and the size of the stigmas.

Type locality: "Province of St. Jago de Tucuman, La Plata," Argentina. *Distribution:* Central Argentina.

Specimens examined: ARGENTINA: La Plata (Tweedie *1191*, the type, a fragment in the Engelmann herbarium); Buenos Aires (Beltfreund and Koester *342*); Cordoba (Galander; Lorentz *90*); Parana (Gilbert *78*); Prov. La Rioja (Hieronymus and Niederlein *745*, *846*).

34. *Cuscuta indecora* Choisy

For the synonymy and description of this species and its varieties see Yuncker, Ill. Biol. Monogr. **6**: 147. 1921; also Progel in Martius, Flora Brasiliensis **7**: 382, Pl. 127, fig. 6. 1871.

KEY TO THE VARIETIES

Scales ovate, prominently fringed.
  Calyx lobes ovate, obtuse or acutish, not exceeding the corolla.
    Styles as long as the ovary, not divaricate.
      Flowers 2–3 mm. long, papillose-hispid (no specimens of this have been seen from South America) ..... *hispidula.*
      Flowers usually larger, not papillose-hispid ..... *neuropetala.*
    Styles short, becoming divaricate in fruit ..... *subnuda.*
  Calyx lobes lanceolate, acute, usually exceeding the corolla ..... *longisepala.*
Scales triangular, shallowly fringed ..... *integruiscula.*
Scales bifid, rudimentary ..... *bifida.*

Cuscuta indecora neuropetala (Choisy) Hitchcock

Distribution in South America: Venezuela, Brazil, and Paraguay.

Specimens examined: BRAZIL: Maranham (Gardner *6068* in part).
PARAGUAY: Asunción (Lindman *A 2325*).
VENEZUELA: Ciudad Bolivar (L. H. and Ethel Zoe Bailey *1255*).

Cuscuta indecora subnuda Engelmann

Cuscuta indecora subnuda Engelmann, Trans. Acad. Sci. St. Louis **1**: 502. 1859.

Engelmann characterizes this variety as having an exserted capsule and short, divaricate styles. I did not see the type specimen nor any other that would seem to belong here. It is apparently a rare form.

Cuscuta indecora longisepala Yuncker

Distribution in South America: Through central part of the continent.

Specimens examined: BOLIVIA: Gran Chaco (Fries *1629*).
PARAGUAY: (Balansa *2062;* Morong *259*).
PERU: Piura (Spruce); Pacasmayo (Mr. and Mrs. J. N. Rose *18517*).

Cuscuta indecora integriuscula Engelmann

Cuscuta indecora integriuscula Engelmann, Trans. Acad. Sci. St. Louis 1: 502. 1859.

Scales shallowly fringed, triangular, reaching the stamens, bridged at about the middle; styles very slender, shorter than the ovary; calyx lobes triangular, acute, and about equal to the corolla tube.

Type locality: Mendoza, Argentina. *Distribution:* Known only from the type locality.

Specimens examined: ARGENTINA: Mendoza (Gillies, the type, a fragment in the Engelmann herbarium).

Cuscuta indecora bifida Yuncker

Distribution in South America: Brazil.

Specimen examined: BRAZIL: Minas Geraes (Gardner *5036*. This specimen has lanceolate calyx lobes characteristic of variety *longisepala* and the bifid scales of this).

35. *Cuscuta globosa* Ridley

(Pl. V, fig. 26, *A–E*)

Cuscuta globosa Ridley, Journ. Linn. Soc. 27: 48. 1890.

Stems slender. Flowers 1.5–2 mm. long, on pedicels mostly exceeding the length of the flowers; calyx about as deep as the corolla, lobes triangular, acute, frequently unequal; corolla campanulate; lobes triangular, acute, upright, about equal to the tube; stamens about equaling the lobes, slenderly subulate filaments much longer than the oval anthers; scales reaching the stamens, ovate, moderately fringed with short processes, bridged below the middle; styles longer than the globose ovary. Capsule depressed-globose, thin, not circumscissile; seeds commonly two in each capsule, about 1–1.25 mm. long, subglobose, flattened on one side, hilum small, withered corolla about the capsule towards its base, the capsule much exserted.

This species seems to be closely related to both *C. acuta* and *C. micrantha*, but differs from both of these species in the possession of pedicels mostly longer than the flowers, in its smaller flowers, and longer stamens. Also, the withered corolla is retained towards the base of the capsule.

Type locality: Island of Fernando de Noronha, Brazil. *Distribution:* Known only from the type locality.

Specimens examined: BRAZIL: Fernando de Noronha, summit of Morro Branco, and near Tangle Bay (Ridley, Lea, and Ramage *72*, taken to represent the type, in the Royal Botanical Museum, Kew).

36. *Cuscuta micrantha* Choisy

Cuscuta micrantha Choisy, Mém. Soc. Phys. Hist. Nat. Genève 9: 271, Pl. 1, fig. 3. 1841; and in De Candolle, Prodromus 9: 453. 1845.—Gay, Fl. Chile 4: 446. 1849.—Engelmann, Trans. Acad. Sci. St. Louis 1: 500. 1859.—Reiche, Fl. Chile 5: 171. 1910.

Cuscuta popayanensis Poeppig, ex Engelmann, Trans. Acad. Sci. St. Louis 1: 500. 1859.

? Cuscuta pauciflora Philippi, Linnaea 33: 185. 1864.

Cuscuta sparsiflora Philippi in sched. ex Reiche, Fl. Chile 5: 171. 1910.

Stems slender. Flowers about 2–3 mm. long, on pedicels shorter than the flowers in rather dense, compact clusters, or looser in the second variety; calyx about as long as the corolla tube; lobes ovate, acute; corolla campanulate, lobes ovate, acute, upright or slightly spreading, shorter than, or (in the second variety) about equaling, the tube; scales narrow, oblong, quite firmly attached for nearly their whole length, sparingly fringed about the top with few processes, bridged at about a quarter of their height, or the scales larger and more prominently fringed and with the bridges somewhat higher, about reaching the stamens; stamens shorter than the lobes, filaments shorter than or exceeding the length of the ovate-cordate to oval anthers, slender or slightly subulate; styles slender and shorter than, or about equal to, the ovoid ovary. Capsule ovoid, or globose and somewhat depressed, hilum small, about 1 mm. long.

Cuscuta micrantha typica

(Pl. I, fig. 2, *A–E*)

Flower clusters small, compact; corolla lobes shorter than the tube, scales narrow, sparingly fringed about the upper portion only, flowers about 2 mm. long, anthers small, ovate-cordate, ovary and capsule ovoid, stigmas small.

Type locality: Prov. Coquimbo, Chile. *Distribution:* Central Chile.

Specimens examined: CHILE: Prov. Coquimbo (Gay *538*, the type, a fragment in the Engelmann herbarium); Santiago (Philippi; Reiche *III–99*); Cerro Blanco (Hastings *154*. This specimen shows characters of this and also of the next variety).

Cuscuta micrantha latiflora Engelmann

(Pl. I, fig. 2, *F*, *G*)

Cuscuta micrantha latiflora Engelmann, Trans. Acad. Sci. St. Louis 1: 500. 1859.—Gay, Fl. Chile 4: 446. 1849.

Cuscuta pusilla Philippi, in herb.

Flowers 2.5–3 mm. long, corolla lobes about equal to the tube, anthers oval and larger than in the preceding variety; scales larger and more prominently fringed. Capsule globose and somewhat depressed, three- or four-seeded.

Type locality: Concon, Chile. *Distribution:* Chile.

Specimens examined: CHILE: (v. Better *142;* this specimen approaches the typica variety; Reed); Concon (Poeppig, taken to represent the type, a fragment in the Engelmann herbarium); Valparaiso (Brenning *91*); Panamavida, Linares (E. W. D. and Mary M. Holway Dec. 17, 1919); Desert of Atacama (Morong *1163*); Sans Jago (Hohenacker *489*).

37. *Cuscuta acuta* Engelmann

(Pl. II, fig. 8, *A–E*)

Cuscuta acuta Engelmann, Trans. Acad. Sci. St. Louis 1: 497. 1859.

Stems slender. Flowers 2–3 mm. long, on pedicels about as long as the flowers, thin and membranous, in umbellate clusters; calyx campanulate, lobes triangular-ovate, acute to acuminate or somewhat cuspidate, or obtusish, as long as, or exceeding, the corolla tube; corolla campanulate, lobes lanceolate, acute to acuminate, upright or somewhat spreading in fruit, as long as, or longer than, the tube, stamens about three fourths as long as the corolla lobes; filaments stout, subulate, about equal in length to, or much longer than, the oval anthers; scales reaching the stamens, thin, bridged below the middle, closely adherent to the tube, fringed about the upper half; styles about equal to the globose ovary, slightly subulate. Capsule not circumscissile though very easily breaking away at the base, carrying the withered corolla about it, very thin and almost transparent in some specimens so that the seeds are visible in it, depressed-globose, intrastylar aperture comparatively large, styles upright or more often becoming divergent; seeds about 1 mm. long, ovate, hilum short, oblong.

This species differs from *C. pentagona*, which it resembles somewhat, in the shapes of the filaments, calyx and corolla lobes, and the divergent styles.

Type locality: "Chatham Island of the Galapagos group." *Distribution:* Galapagos Islands.

Specimens examined: Galapagos Islands: Chatham Island (Andersson, the type, a fragment in the Engelmann herbarium); South Marborough Island (Snodgrass and Heller *318*); Bindloe Island (Snodgrass and Heller *769*).

38. **Cuscuta insquamata** n. sp.

(Pl. V, fig. 30, *A–D*)

Stems slender. Flowers 1.5–2 mm. long, delicate, 4- or 5-parted, on pedicels shorter than or longer than the flowers in dense cymose clusters, membranous with a few scattering yellow, pellucid glands; calyx longer than the corolla tube, lobes triangular, acuminate; corolla shallowly campanulate, lobes upright, triangular, acuminate, about as long as the tube; stamens shorter than the lobes, ovate anthers about equal to the stout, subulate filaments; scales lacking; slender styles shorter than, or equaling, the globose, somewhat pointed ovary, which is thickened at the apex. Capsule carrying the corolla about it towards the base, depressed-globose, with a fleshy collar about the intrastylar aperture, evidently not circumscissile although a few capsules seemed to have a weaker zone towards the base where they break loose when pulled; seeds four in each capsule, roundish, hilum small.

Type locality: Bolivia. *Distribution:* Known only from the type locality.

Specimens examined: Bolivia: (Fiebrig *3045*, the type, in the Museum of Natural History, Asunción, Paraguay, a fragment in the author's herbarium).

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——. *C. odorata typica*
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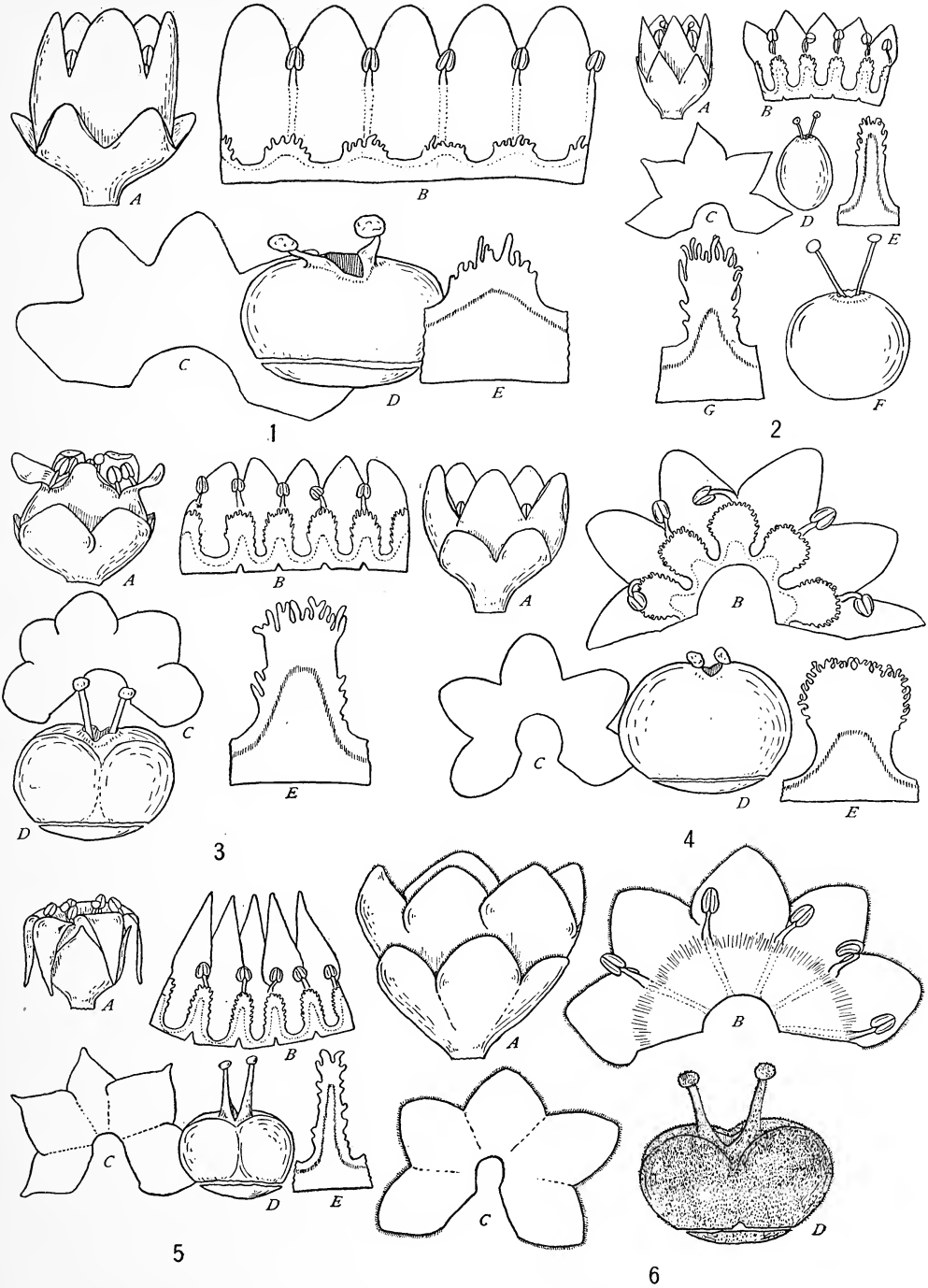
EXPLANATION OF PLATES

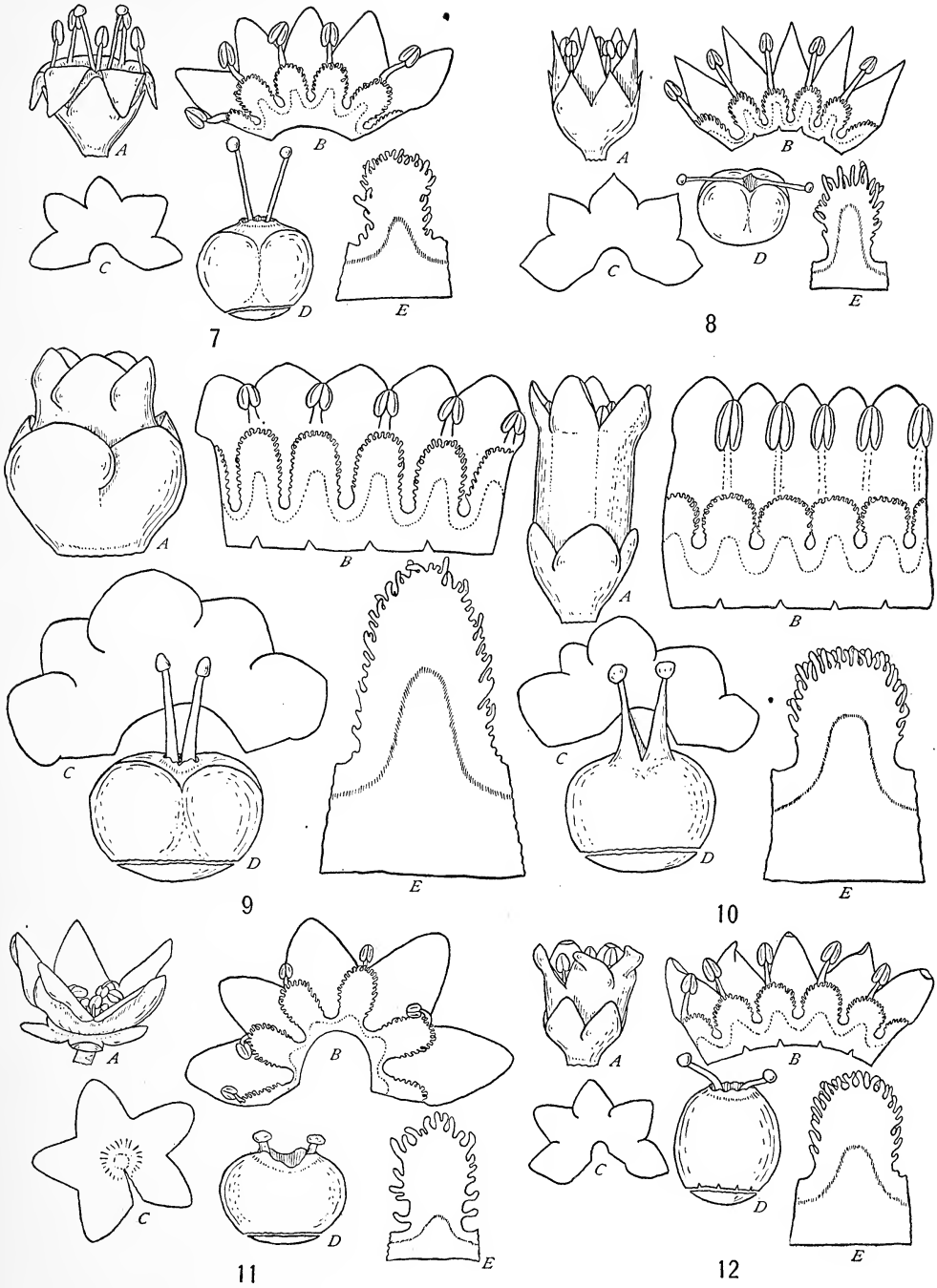
All figures are × 5 except those representing the individual scales which are × 10.

PLATE I

FIG. 1, *A–E*. *Cuscuta brevisquamata*, the type collection.
FIG. 2. *Cuscuta micrantha: A–E*, var. *typica*, the type collection; *F, G*, var. *latiflora*.
FIG. 3, *A–E*. *Cuscuta boliviana*, the type collection.
FIG. 4, *A–E*. *Cuscuta microstyla* (Fries *906*).
FIG. 5, *A–E*. *Cuscuta acutiloba*.
FIG. 6, *A–D*. *Cuscuta grandiflora*.



YUNCKER: SOUTH AMERICAN SPECIES OF CUSCUTA



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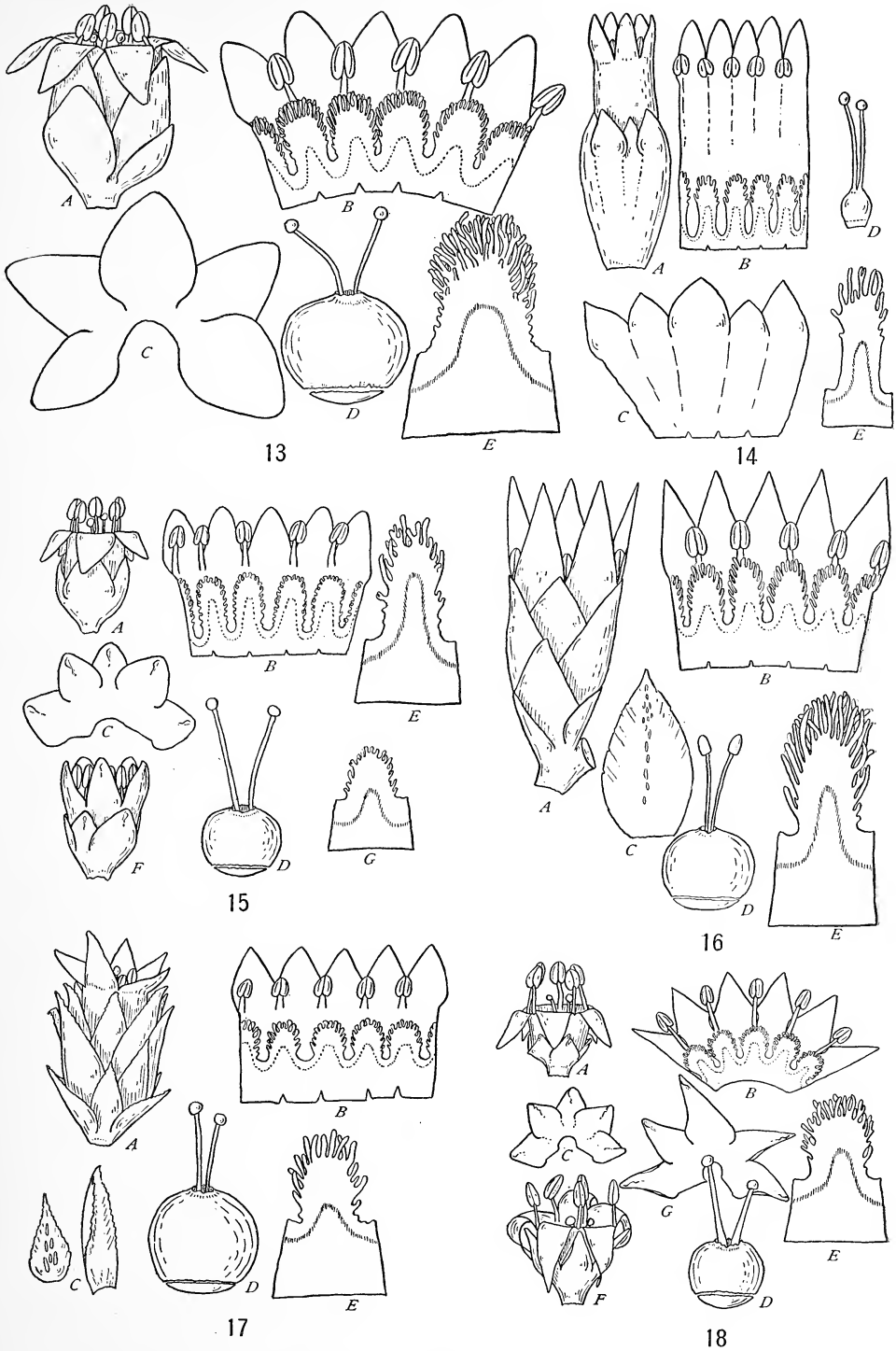

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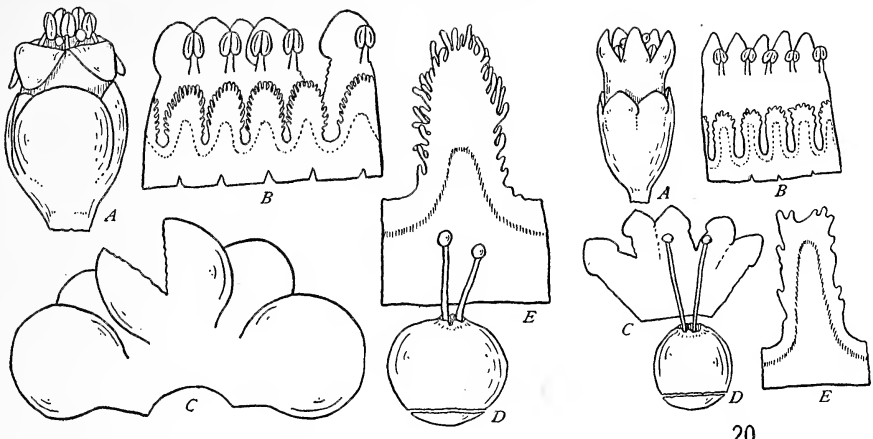

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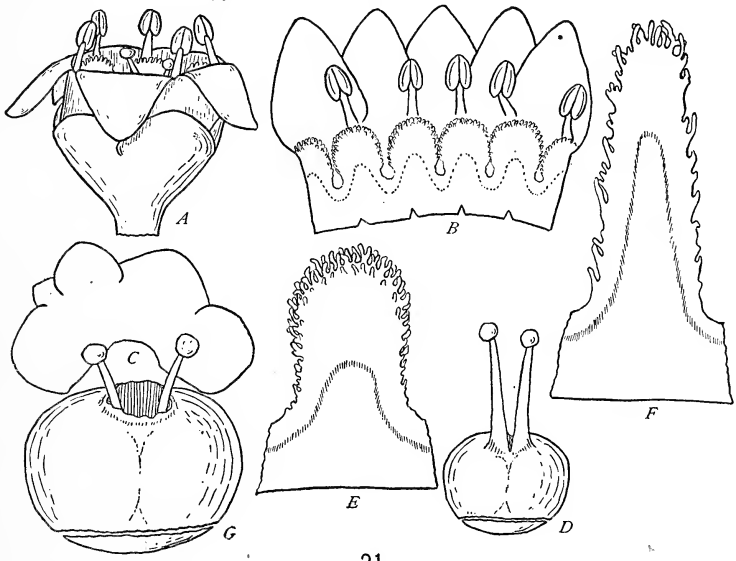

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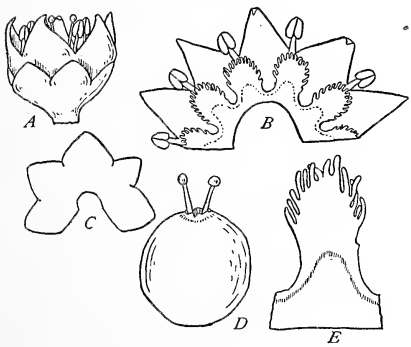

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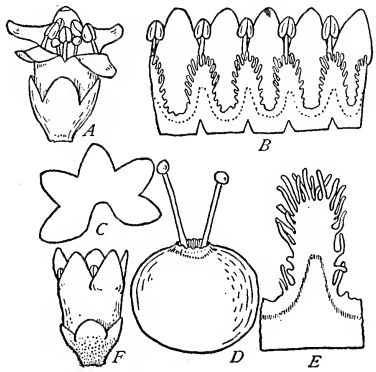

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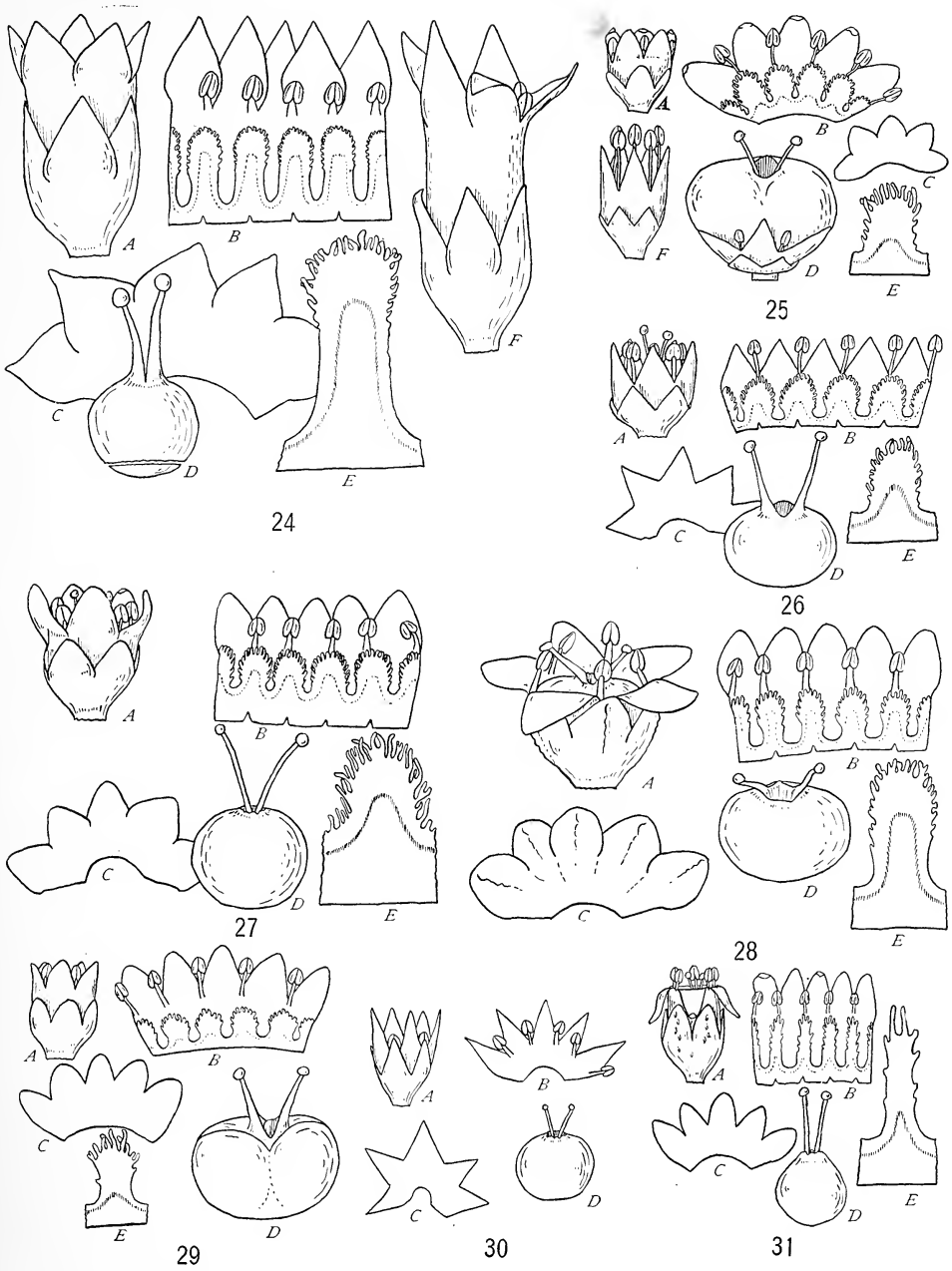
YUNCKER: SOUTH AMERICAN SPECIES OF CUSCUTA



YUNCKER: SOUTH AMERICAN SPECIES OF CUSCUTA

Plate II

Fig. 7, *A–E*. *Cuscuta incurvata*.
Fig. 8, *A–E*. *Cuscuta acuta*, the type collection.
Fig. 9, *A–E*. *Cuscuta globiflora*.
Fig. 10, *A–E*. *Cuscuta chilensis*.
Fig. 11, *A–E*. *Cuscuta argentinana*, the type collection.
Fig. 12, *A–E*. *Cuscuta corniculata*.

Plate III

Fig. 13, *A–E*. *Cuscuta goyaziana*, the type collection.
Fig. 14, *A–E*. *Cuscuta prismatica*, the type collection.
Fig. 15. *Cuscuta trichostyla: A–E*, var. *typica*, the type collection; *F*, *G*, var. *carinata*, the type collection.
Fig. 16, *A–E*. *Cuscuta bracteata*, the type collection.
Fig. 17, *A–E*. *Cuscuta serrata*, the type collection.
Fig. 18. *Cuscuta xanthochortus: A–E*, var. *typica; F*, *G*, var. *lanceolata*, the type collection.

Plate IV

Fig. 19, *A–E*. *Cuscuta orbiculata*, the type collection.
Fig. 20, *A–E*. *Cuscuta corymbosa microlepis*, the type collection.
Fig. 21. *Cuscuta odorata: A–E*, var. *typica; F*, var. *Holwayana*, the type collection; *G*, var. *botryoides*, the type collection.
Fig. 22, *A–E*. *Cuscuta gymnocarpa*.
Fig. 23. *Cuscuta racemosa: A–E*, var. *typica; F*, var. *miniata*.

Plate V

Fig. 24. *Cuscuta foetida: A–E*, var. *typica; F*, var. *pycnantha*, the type collection.
Fig. 25. *Cuscuta parviflora: A–E*, var. *typica; F*, var. *elongata*.
Fig. 26, *A–E*. *Cuscuta globosa*, the type collection.
Fig. 27, *A–E*. *Cuscuta platyloba*.
Fig. 28, *A–E*. *Cuscuta cristata*.
Fig. 29, *A–E*. *Cuscuta obtusiflora*, the type collection.
Fig. 30, *A–D*. *Cuscuta insquamata*, the type collection.
Fig. 31, *A–E*. *Cuscuta stenolepis*, the type collection.

A COMPARATIVE STUDY OF SAND AND SOLUTION CULTURES OF MARQUIS WHEAT

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The present study, in which a comparison is made of the growth of Marquis wheat in sand and solution cultures, represents one of several experiments involving solution III of the National Research Council series which are now in progress in the plant-physiology laboratory at Iowa State College.

Most investigators attempting to determine the salt requirements of higher plants have used water cultures. Tottingham (25) placed the whole proposition on a definite quantitative basis. He took the well-known Knop's solution, and, by varying the proportions of salts under definite volume-molecular partial concentrations, procured a superior yield of wheat of 11 percent over the original solution.

Shive (19) followed the plan suggested by Tottingham[1] and, making use of a three-salt solution containing mono-potassium phosphate, calcium nitrate, and magnesium sulphate, obtained an increase in yield of wheat of 27 percent over Tottingham's solution of the same total concentration. McCall (10) used Shive's solution and grew wheat in sand cultures. He noted that the average dry weights of both tops and roots were decidedly greater for the plants grown in sand than for those grown in the solution cultures. Incidentally there was a marked difference between the solutions producing the highest yield of plants in sand and those giving the best growth in Shive's cultures.

Livingston and Tottingham (8) made a preliminary test of a series of type III solutions, containing the three component salts KNO_3, $Ca(H_2PO_4)_2$, and $MgSO_4$. Their results showed that the solution IIIR6C1 was apparently just as good as Shive's best IR5C2 solution. However, this experiment lasted only 18 days.

Shive (20) and Shive and Martin (22) studied the salt requirements of young and of mature buckwheat plants in both solution and sand cultures. They found that the solution yielding the maximum weight of tops during the early developmental period, and the solution that gave the highest yield of tops and of roots during the late period of development, were identical for both the solution and the sand cultures, but the actual dry weight of

[1] Tottingham (25) has presented an extensive review of literature covering the subject of solution cultures. For citations see pages 242–245.

the plants was considerably greater for the sand cultures than for the solution cultures.

Meier and Halstead (14) ran a series of wheat experiments with Shive's three-salt solution, each series comprising 21 cultures of different salt proportions all having an osmotic concentration of one atmosphere. They found that no one culture gave consistently high yields of plants, and noted, as did Livingston (6), Shive (19), McCall (10), Wolkoff (27), and others, that the total amount of transpiration is as good a criterion as is the final dry weight of plants for studying the comparative growth obtained in different solutions.

Bouyoucos (1) observed that the amount of transpiration per gram of dry matter tended to be larger in the sand than in solution cultures receiving the same densities of solution, while the actual dry matter produced was greater in the solution than in the sand cultures. Lyon and Bizzell (9) found that wheat seedlings growing in crushed quartz containing the same nutrient solutions as those used in water cultures produced the same relative quantities of dry matter per unit of transpiration as did the water cultures, but the quantitative production of dry matter per unit of transpiration was in each case greater in the quartz than in the water cultures.

Certain investigators, McCall (10), Lyon and Bizzell (9), and others, claim that the superiority of sand cultures over solution cultures is due primarily to the adsorptive properties of the sand particles. Wolkoff (27) concluded from his work that adsorption was not the factor which modified the concentration of the solution to such an extent as to cause even slight differences in plant growth. Shive (21) also found no evidence of the adsorption of salts or ions in sufficient amounts to change the total concentration of the solutions.

In recent years considerable attention has been devoted to studying the effect of the reaction of the medium of sand and water cultures on the growth of plants. Hoagland (4) studied the effect of hydrogen- and hydroxyl-ion concentration on barley seedlings growing in partial nutrient solutions of like osmotic pressure. He noted that the OH ion was more toxic than the H ion for similar divergencies from the neutral point. Later the same author (5) found that an acid solution (pH 5) was not injurious to the barley plant at any period in its growth. As in his previous work, he observed a tendency on the part of the plant to adjust the reaction of the medium toward the neutral point. Toole and Tottingham (24) also noted that barley seedlings had a marked neutralizing effect on the nutrient solution, and that the solution in which the plants had been grown showed a more nearly uniform hydrogen-ion concentration than did the original solution. They found that the weights of dry tops of barley were inversely proportional to the hydrogen-ion concentration of the solution.

Duggar (2) concluded from his work that the tendency for the shifting of an acid reaction toward the neutral point depends in part upon the

composition of the solution and in part upon the plant grown. Shive (21) demonstrated that the reaction of a nutrient solution is not noticeably changed by being in contact with sand. Salter and MacIlvaine (15) studied the effect of reaction of solution on germination and on growth of seedlings of wheat, soybeans, corn, and alfalfa. The maximum growth of wheat, soybeans, and alfalfa was obtained in a solution having a reaction of 5.94 pH. The maximum growth for corn was obtained at 5.16 pH. These authors noted that the OH ion is more harmful than the hydrogen ion in equivalent concentrations. Meier and Halstead (14) reported that there is no correlation between the yield of plants and the hydrogen-ion concentration or the change in pH. McCall and Haag (13) varied the hydrogen-ion concentration in different nutrient solutions, and noted that the hydrogen-ion concentration has a marked effect upon the rate of growth of the wheat plant and is an important factor in the control of chlorosis.

The object of the work reported in this paper was to determine the best proportion of the three salts KNO_3, $Ca(H_2PO_4)_2$, and $MgSO_4$ for the growth of wheat, and also to compare the sand- and water-culture methods with respect to transpiration, total weight of tops and roots, and the reaction of the media as determined by means of the hydrogen electrode.

TABLE 1. *Partial Volume-molecular Concentrations and Molecular Proportions of KNO_3, $Ca(H_2PO_4)_2$, and $MgSO_4$ in 21 Solutions all having an Osmotic Value of Approximately 1.00 Atmosphere at 25°C. but Differing by Increments of One Eighth in the Salt Proportions*

Solution Number	Molecular Proportions			Partial Volume-molecular Concentrations		
	KNO_3	$Ca(H_2PO_4)_2$	$MgSO_4$	KNO_3	$Ca(H_2PO_4)_2$	$MgSO_4$
III						
R1S1	1	1	6	.0027	.0027	.0165
S2	1	2	5	.0026	.0053	.0132
S3	1	3	4	.0024	.0073	.0098
S4	1	4	3	.0023	.0093	.0070
S5	1	5	2	.0021	.0106	.0042
S6	1	6	1	.0021	.0125	.0021
R2S1	2	1	5	.0054	.0027	.0135
S2	2	2	4	.0048	.0048	.0096
S3	2	3	3	.0045	.0067	.0067
S4	2	4	2	.0042	.0084	.0042
S5	2	5	1	.0041	.0103	.0020
R3S1	3	1	4	.0075	.0025	.0090
S2	3	2	3	.0070	.0047	.0070
S3	3	3	2	.0067	.0067	.0045
S4	3	4	1	.0064	.0086	.0021
R4S1	4	1	3	.0099	.0025	.0074
S2	4	2	2	.0093	.0047	.0047
S3	4	3	1	.0085	.0064	.0021
R5S1	5	1	2	.0125	.0024	.0048
S2	5	2	1	.0113	.0045	.0023
R6S1	6	1	1	.0139	.0023	.0023
Shive's R5C2 (1.75 atm.)	3.77	1.09	3.14	.0180	.0052	.0150

Experimental

The plan of the experiment was in all its essential details the same as that recommended by the Special Committee of the National Research Council on Salt Requirements of Agricultural Plants (23).

The salts used were especially prepared for this work at the request of this committee by the J. T. Baker Chemical Company. The molecular proportions and partial volume-molecular concentrations of the three salts, KNO_3, $Ca(H_2PO_4)_2$, and $MgSO_4$, are given in table 1. These dilutions are based on freezing-point determinations made by Dr. Shive.

The wheat used was of the "Marquis" variety, secured through Dr. Stakman of the University of Minnesota. In the matter of securing uniform seedlings of 4 cms. length, the seeds were first soaked in a germinating solution (Shive's R5C2, 0.1 optimal concentration), and later distributed evenly upon a germinating net made by taking ordinary mosquito netting which had been thoroughly paraffined; this net was tied tightly over a 5-gallon glazed stoneware jar. Provision was made so that two liters of fresh germinating solution could be added from the bottom of the container each day. In order to maintain a temperature of approximately 18° C., the jar was kept in a water bath. It then became a simple matter to secure the seedlings of desired uniformity. For the water cultures the seedlings were mounted in cork stoppers to fit 1-quart Mason jars in the manner adopted by Tottingham. Five plants were used for each culture.

The sand used was obtained from the Clayton Sand Company of Clayton, Iowa, and gave a mechanical analysis as follows:

Millimeters in Diameter				
1.00–0.5	0.5–0.25	0.25–0.10	0.10–0.05	0.05–0.00
0.06%	62.80%	14.12%	21.40%	1.62%

A chemical analysis showed the sand to contain 98.5 percent SiO_2 and 0.54 percent iron and aluminum hydroxide. It had a water-holding capacity of 21 percent on the dry-weight basis. [Hilgard method with column 1 cm. high (3).]

Regarding the optimum moisture content of a soil as 50 percent of its maximum water-holding capacity, the sand cultures were maintained at a moisture content of 11 percent.

After the experiment had been in progress for one month, it was realized that the 11 percent moisture was not sufficient to supply the needs of maximum transpiration, and consequently the moisture content was raised to 16 percent for the remaining period of experimentation.

Glazed stone or butter jars 12.5 cm. high and 19.0 cm. in diameter were used for these cultures. A glass suction tube, the lower end of which was

loosely plugged with glass wool, was vertically placed against the wall of the jar. Its lower end rested on the bottom of the jar and the upper end extended just above the rim. The tube was held in place while the sand was added. Each jar was filled with 1250 grams of carefully washed sand.

For the supply orifice, 100-cc. wide-mouthed bottles with the bottoms removed were used. Each bottle rested on an inverted glazed porcelain dish according to the method of McCall and Richards (11). After five seedlings were transferred to the sand medium, enough solution was added through the mouth of the bottle to bring the liquid to about 1 cm. above the sand, and the crock was slightly jarred to settle the sand about the roots of the seedlings and to give a level surface for the wax seal (80 parts paraffin, 20 parts vaseline). The outlet tube was then connected with a vacuum pump, and the excess solution was drawn off. The sand was flooded again, and suction was applied until the moisture content was optimum.

When the seedlings were in place the total weights of cultures were recorded. All solutions were renewed twice a week throughout the continuance of the experiment. At the time, the cultures were weighed and the amount of water lost by transpiration was recorded.

The growth period extended from January 15, 1921, to March 24, 1921, which brought the plants to the stage at which the advanced cultures were just beginning to head.

Determination of Hydrogen-Ion Concentration

After the plants were harvested from the sand and water cultures, samples were taken from each culture for the purpose of determining the hydrogen-ion concentration of the medium after the plants had grown in it for one $3\frac{1}{2}$-day period.

The apparatus used in this work included the following pieces obtained from the Leeds and Northrup Company: potentiometer, 3-dial resistance box, Wall d'Arsonval galvanometer (type P), Eppley standard cell, single-contact key, one double-throw switch, and two dry-cell batteries. An Ostwald normal calomel electrode and a Hildebrand hydrogen-gas electrode were used. The hydrogen, obtained from a tank of compressed hydrogen, was purified by bubbling through a 5-percent solution of potassium permanganate, then through a 5-percent solution of pyrogallic acid, and finally through distilled water. The hydrogen-electrode vessel used was similar to the one described by Sharpe and Hoagland (18), consisting of a small wide-mouthed bottle of 75 cc. capacity. This was fitted with a rubber stopper into which were inserted the hydrogen electrode, a small exit tube, and a small bent glass tube filled with agar jelly saturated with KCl. Connection was made with the calomel cell by means of a beaker containing a saturated KCl solution and the agar tube.

The solution or suspension to be tested was placed in the hydrogen-

electrode vessel and hydrogen gas was bubbled through for several minutes. The exit tube was then closed and the vessel was shaken for two minutes, after which processes a reading was made. These processes were repeated until a constant reading was obtained. The voltmeter readings were transformed into pH values from the tables prepared for this purpose by Schmidt and Hoagland (16).

Results

When the more advanced cultures were just beginning to head out, each culture, containing five plants, was harvested as a whole in the usual manner. The tops were placed in weighed beakers, and the green weights were recorded. They were then dried to constant weight in an electric oven. Likewise, the roots were dried in the same manner and weighed. The roots of the sand cultures were first thoroughly washed free from sand.

The data for total absorption, fresh and dry weights of tops, and the dry weight of roots for the sand and water cultures are given in table 2. The five sand and water cultures showing the greatest transpiration, the highest fresh weight of tops, and the largest dry weight of tops and roots are marked *H*. Likewise the five lowest cultures in each case are marked *L*.

TABLE 2. *Total Absorption for the Growth Period, the Fresh Weight of Tops, and the Dry Weight of Tops and Roots, of Water and Sand Cultures*

Solution Number	Total Absorption (grams)		Fresh Wt. of Tops (grams)		Dry Weight			
					Tops (g.)		Roots (g.)	
	Water	Sand	Water	Sand	Water	Sand	Water	Sand
III (1.00 atm.)								
R1S1	4866	3685	44.6	24.1	9.786H	5.461	2.741H	5.998
S2	4254	3655	37.8	24.5	7.626	5.731	2.951H	7.006H
S3	3174	1774L	32.5	7.9L	5.748	1.693L	1.996	2.183L
S4	382L	2248L	2.5	12.1L	0.125L	2.597L	0.198L	3.947
S5	167L	1576L	0.7L	4.3L	0.025L	1.364L	1.146L	22.672L
S6	91L	998L	0.1L	1.9L	0.041L	0.857L	0.082L	1.869L
R2S1	**6203H**	3935	**65.5H**	31.7	**15.828H**	6.045	2.730	6.678H
S2	4647	4078	48.0	33.2	12.860H	6.992	2.490	**12.547H**
S3	3997	3526	47.0	37.5	7.126	5.398	2.454	7.254H
S4	1503	3882	4.5	29.9	1.677	7.262	0.493	6.360
S5	120L	2730L	0.3L	18.3L	0.140L	3.945L	0.138L	3.589
R3S1	4922	3778	47.8	36.3	10.147H	5.857	2.507	4.391
S2	5825H	4312	62.7H	36.4	9.440H	7.842H	**3.867H**	7.461H
S3	3430	4499H	38.0	43.2H	4.369	**8.992H**	1.992	5.740
S4	288L	4253	1.0L	34.3	0.275L	7.389	0.222L	4.894
R4S1	4936	4459H	55.1H	32.6	8.861	5.462	2.413	1.858L
S2	4905	4578H	55.0H	**50.0H**	7.980	7.880H	3.675H	3.648
S3	518	4377H	1.9L	45.2H	0.396	8.665H	0.244	3.215L
R5S1	5207H	4254	60.0H	38.3	9.050	6.142	2.260	5.261
S2	5306H	**4707H**	53.2	48.2H	6.726	7.981H	2.839H	2.904
R6S1	5063H	4305	52.0	38.5H	7.593	6.123	1.663	2.476
Shive's R5C2 (1.75 atm.)	3147	3514	34.2	26.6	5.563	4.695	2.582	2.805
	3669	3323	23.0	20.7	7.365	4.141	3.355	2.526

Considering first the absorption data presented in table 2, it will be seen that the amount of water absorbed or lost by transpiration from the water cultures is very much greater, with few exceptions, than it is from the sand cultures. It is interesting to note that the sand cultures transpiring more water than the water cultures are characterized by having a high proportion of $Ca(H_2PO_4)_2$ with only a small proportion of KNO_3 and $MgSO_4$. The five water cultures showing the greatest absorption (marked *H*) are not the same as the five sand cultures showing the greatest absorption, with the exception of culture R5S2. The five cultures showing the lowest absorption are the same for both sand and water cultures with but a single exception. In the case of the control solutions, the quantity absorbed is practically the same for both sand and water cultures. However, the total absorption for the control solutions is very much lower than that for the five sand and water cultures marked *H*. These transpirational data, therefore, show a marked superiority of type III solution over Shive's "best" solution (R5C2—1.75 atm.).

Three of the five water cultures showing the greatest absorption also have the highest fresh and dry weight of tops, while four of the five sand cultures marked *H* reveal this relation. Thus, in general these data uphold the conclusion of Livingston (6) and of other writers that the amount of transpiration appears to be as good a criterion as the final dry weight for judging the comparative growth obtained in different solutions.

A comparison of the fresh and dry weight of tops produced by the sand and water cultures shows that the highest yields favor the water cultures with the exception of those cultures having high concentrations of $Ca(H_2PO_4)_2$. But the sand cultures favor the greatest growth of roots, as shown by a comparison of the dry weight of roots grown in the sand and water cultures. By comparing the dry weight of tops produced by the high-yielding sand and water cultures with the average dry weight of tops produced by the control solution (Shive's R5C2), it will be seen that the water culture IIIR2S1 is superior to Shive's R5C2 by 144 percent, while the sand culture IIIR3S3 is superior to Shive's R5C2 by 103 percent. When the dry weight of roots of IIIR3S2 of water cultures is compared with the dry weight of roots of Shive's R5C2 there is a difference of 30 percent. These differences are great enough to overbalance any possible errors in plant variation, and furnish conclusive evidence that the optimum proportion of salts in type III solution gives better growth for wheat than Shive's (R5C2—1.75 atm.) solution.

In comparing the data given in table 4, it is clear that there is very little difference in the total dry weight (tops and roots) of the sand and water cultures. On the total dry-weight basis, the average increased yield of the best sand and water cultures of type III solution over the average yield of Shive's solution is 131 percent.

As a means of facilitating comparison of the salt requirements of wheat

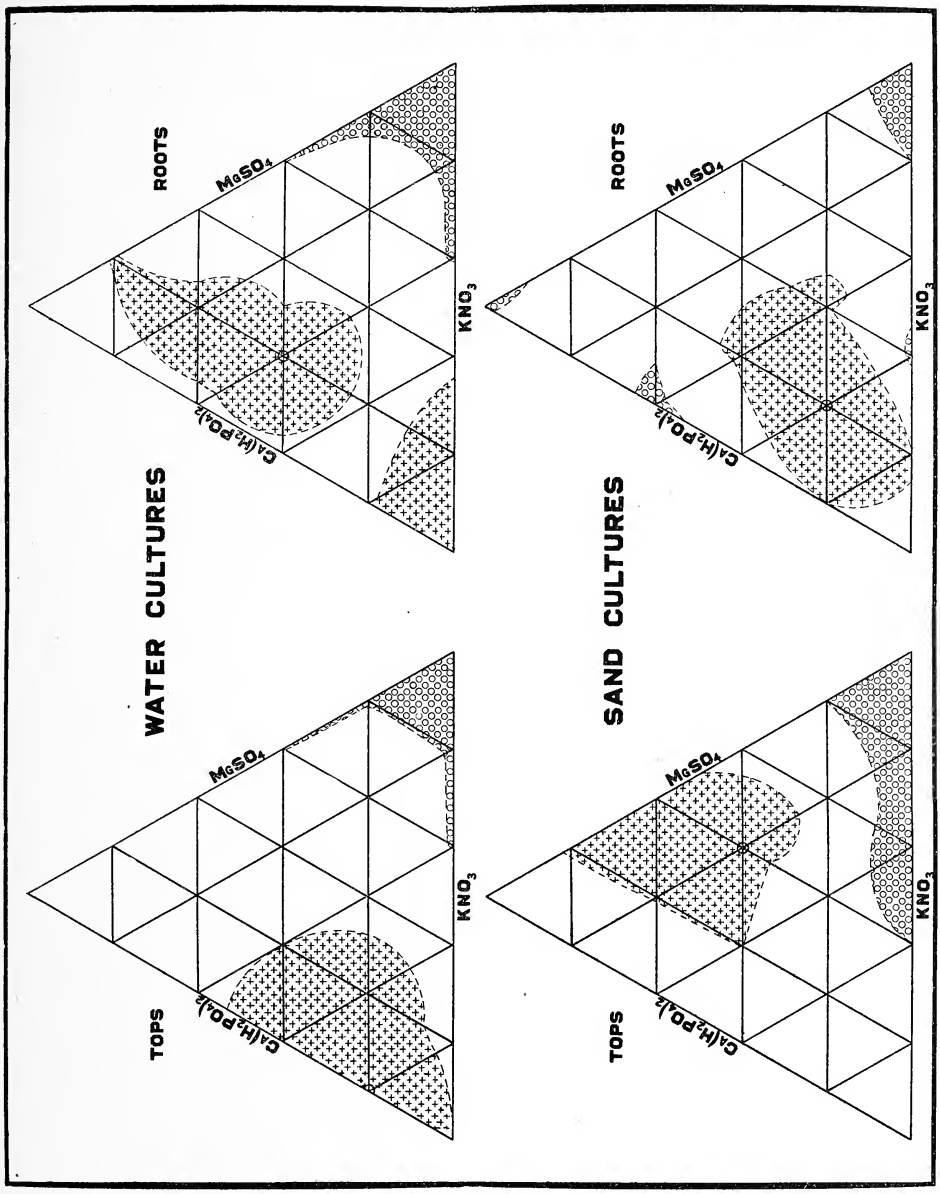

FIG. 1. Diagrams showing relative yields of tops and roots.

when grown in sand and water cultures, use has been made of the triangular diagram in discussing these results. This scheme was first suggested for this kind of work by Schreiner and Skinner (17) and was later modified and perfected by Tottingham (25), Shive (19), McCall (10), and Trelease (26). The details of the arrangement of the cultures in the triangle are given by all of the above-named authors and also by the plan of the committee (23). The triangular diagrams for the dry weights of tops and roots for water and sand cultures are shown in figure 1. The five cultures lying in the high-yielding areas, marked *H* in table 2, are plotted on the triangles with small + signs. Similarly, the five cultures lying in the low-yielding areas, marked *L* in table 2, are plotted on the triangles with small circles. This method was suggested by Shive (19). The culture having the highest yield in each diagram is marked with a circle.

In figure 1, comparing first the triangles representing the dry weight of tops of wheat plants grown in water and sand cultures, the five high-yielding water cultures (15.82–9.44) occupy the lower left position of the triangle, whereas the five high-yielding sand cultures (8.99–7.84) assume the upper right central portion of the triangle. Thus it is seen, as noted by McCall (10), that the water cultures and sand cultures show a marked difference in the salt proportions that are required to produce their respective maximum yields of tops. At only one point, that of R3S2, do the areas overlap, and this is the lowest culture of the five in both cases. The highest-yielding water culture (R2S1) has the following molecular proportions: 2, KNO_3; 1, $Ca(H_2PO_4)_2$; and 5, $MgSO_4$. The partial volume-molecular concentrations are 0.0054 KNO_3, 0.0027 $Ca(H_2PO_4)_2$, and 0.0135 $MgSO_4$. In other words, the solution is made with 18.90 cc. (1.0 M) KNO_3, 94.5 cc. (0.1 M) $Ca(H_2PO_4)_2$, 47.25 cc. (1.0 M) $MgSO_4$, and 3 mg. $FePO_4$, made up to 1 liter with distilled water. The highest-yielding sand culture (R3S3) has the following molecular proportion: 3, KNO_3; 3, $Ca(H_2PO_4)_2$; and 2, $MgSO_4$. The partial volume-molecular concentrations are 0.0067 KNO_3, 0.0067 $Ca(H_2PO_4)_2$, and 0.0045 $MgSO_4$. This solution is made with 23.45 cc. (1.0 M) KNO_3, 234.5 cc. (0.1 M) $Ca(H_2PO_4)_2$, 15.75 cc. (1.0 M) $MgSO_4$, and 3 mg. $FePO_4$ made up to 1 liter with distilled water.

The five low-yielding water cultures (0.125–0.412) occupy the extreme lower right apex of the triangle, and the same is true for the five low-yielding sand cultures (0.856–3.94).

A comparison of the triangles representing the dry weight of roots of the sand and water cultures also shows a wide difference in salt proportions, though not as great as in the case of the tops. Four of the five high-yielding water cultures (3.86–2.74) are located on the line of cultures deriving two eighths of their total molecular proportions from $Ca(H_2PO_4)_2$. The highest-yielding culture (marked with a circle) is characterized by having two eighths of its total diffusion tension due to $Ca(H_2PO_4)_2$; three eighths due to KNO_3; and three eighths due to $MgSO_4$. The area of the high-yielding

sand cultures (12.54–6.67) is found in the lower left central portion of the triangle.

The position of the low-yielding root cultures of the water series is identical with that of the low-yielding top cultures, but this relation does not hold true, at least under the present experiment, for the sand-culture series.

Climatic Conditions

In order that environmental conditions might be as uniform as possible, the cultures were placed upon two revolving tables. The water cultures occupied one and the sand cultures the other.

The following aërial records were secured for the entire growth period: (1) temperature; (2) evaporating power of the air; (3) amount of radiant energy absorbed; and (4) number of hours of sunshine.

Temperature records were obtained from a thermograph placed near the revolving tables. The evaporating power of the air was measured by means of white and black standardized spherical porous-cup atmometers (Livingston, 7). The atmometers were weighed after each $3\frac{1}{2}$-day interval and cleaned once a week. The amount of radiant energy (Livingston, 7) absorbed was determined by the difference in the losses between the corrected readings of the black cup and the readings of the white cup. The duration of sunshine data was kindly furnished by Mr. Charles D. Reed of the United States Weather Bureau office at Des Moines, Iowa. The data relating to environmental conditions are given in table 3.

TABLE 3. *Weekly Averages of Climatic Conditions for the Growth Period*

Date Week Ending	Evaporating Power of Air		Radiant Energy Absorbed	Sunshine	Temperature		
	Loss White Sphere	Loss Black Sphere			Average Maximum	Average Minimum	Mean
	G.	G.	G.	Hours	°C.	°C.	°C.
January 24	82.7	105.8	23.1	38.6	23.1	12.9	18.0
31	64.2	76.8	12.6	18.5	22.0	12.1	17.1
February 7	59.8	76.9	17.1	27.1	23.2	12.6	17.9
14	69.5	94.6	25.1	33.6	21.7	13.2	17.5
21	84.5	120.4	35.9	71.2	25.0	10.8	17.9
28	74.8	102.3	27.5	48.2	30.0	16.7	23.4
March 7	70.4	80.3	9.9	45.5	27.6	13.9	20.8
14	64.2	105.8	41.6	33.7	25.8	14.8	20.3
21	92.4	135.0	42.6	72.6	31.7	12.9	22.3
24	36.1	49.0	12.9	14.4	26.8	18.1	22.5
Total	698.6	946.9	248.3	403.4	——	——	——
Daily average	10.1	13.7	3.6		25.5	13.6	19.6

An examination of the data presented in table 3 shows that the plants

were grown during a period of very unfavorable climatic conditions. There was an unusual amount of cloudiness, as is very evident from the total number of hours of sunshine and the correspondingly low absorption of radiant energy. The mean temperature for the growth period was also lower than that regarded as optimum for the growth of wheat. Under more favorable growing conditions it is possible that the results herein reported would be different.

The data for the hydrogen-ion concentration of type III solutions before growing cultures are taken from the work of McCall and Haag (12). The data for the hydrogen-ion concentration determinations of the solutions after the plants had been grown in them for the last $3\frac{1}{2}$-day period, and the total dry weight per culture for the sand and water cultures, are given in table 4.

TABLE 4. *Showing Hydrogen-ion Concentration of Type III Solutions before Growing Wheat, after the Last $3\frac{1}{2}$-day Interval, and the Total Dry Weight of Tops and Roots of Sand and Water Cultures*

Solution Number	Before Growing Cultures	After Growing Cultures		Total Dry Weight Tops and Roots	
		Water	Sand	Water	Sand
III	pH	pH	pH	Grams	Grams
R1S1	4.1	5.98	6.79	12.527H	17.459H
S2	3.7	5.32	6.72	10.577	12.737
S3	3.6	5.05	6.64	7.744	3.876L
S4	3.6	4.39	6.34	0.323L	6.544L
S5	3.6	4.83	6.08	0.171L	4.036L
S6	3.5	3.65	6.12	0.123L	2.726L
R2S1	4.1	7.20	6.99	**18.558H**	12.723
S2	3.7	5.90	6.81	15.350H	**19.539H**
S3	3.6	5.56	6.74	9.580	12.652
S4	3.6	5.07	5.98	2.170	13.622H
S5	3.5	5.12	5.88	0.278L	7.534
R3S1	4.1	7.20	7.03	12.654H	10.248
S2	3.7	6.98	6.88	13.307H	15.303H
S3	3.6	5.74	6.50	6.361	14.732H
S4	3.6	5.24	6.44	0.497L	12.283
R4S1	4.1	6.98	7.06	11.274	7.320L
S2	3.7	6.57	7.15	11.655	11.528
S3	3.6	5.90	6.49	0.640	11.880
R5S1	4.1	7.08	7.33	11.310	11.403
S2	3.7	6.94	6.91	9.565	10.885
R6S1	3.9	7.92	6.98	9.256	8.599
Shive's R5C2	4.4	5.54	6.30	8.145	7.500
(1.75 atm.)		5.41	6.47	10.720	6.667

The data presented in table 4 show that before growth this solution had a rather acid reaction. After being in contact with the plants for only $3\frac{1}{2}$ days there was a decided change in the hydrogen-ion concentration.

The approach toward neutrality is more striking for the sand cultures than it is for the solution cultures. This statement is particularly true of the control solutions. In the control water cultures the reaction had changed after $3\frac{1}{2}$ days to the extent of 1.0 pH toward neutrality, whereas in the sand cultures it changed from 4.4 pH to 6.38 pH, or a decrease in acidity of 2.0 pH. These data would seem to lend support to the adsorption theory, in that the sand particles apparently have adsorbed sufficient anions from the solutions to cause these differences in pH values, and hence favor greater root development in the sand cultures. The water cultures giving the greatest dry weight of tops and of roots have pH values ranging from 5.90 to 7.20. The sand cultures having the largest total dry weight of tops and of roots have pH values ranging from 5.98 to 6.88. However, an examination of these data in table 4 shows no correlation between total yield of dry matter and the hydrogen-ion concentration.

Summary and Conclusions

The work reported in this paper has offered a means of comparison of sand and water cultures of Marquis wheat when grown in nutrient solutions of type III. Each series of solutions contained 21 cultures of different salt proportions, varied in increments of $\frac{1}{8}$ and all having a total osmotic concentration value of 1.00 atmosphere. The salts used were KNO_3, $Ca(H_2PO_4)_2$, and $MgSO_4$, together with a "trace" of $FePO_4$. The main conclusions to be drawn from this study are as follows:

1. The solutions producing the maximum yield of tops for the sand and water cultures showed marked variations in salt proportions. The "best" water-culture solution (R2S1) was characterized by having two eighths of its total osmotic concentration derived from KNO_3; one eighth from $Ca(H_2PO_4)_2$; and five eighths from $MgSO_4$. The "best" sand-culture solution (R3S3) had three eighths of its total osmotic concentration due to KNO_3; three eighths to $Ca(H_2PO_4)_2$; and two eighths to $MgSO_4$.

2. The high-yielding water culture R2S1 was far superior to that in Shive's R5C2 solution. The high-yielding sand culture also gave much greater production than the control solution.

3. The largest amount of absorption and the maximum green and dry weight of tops favored the water cultures. The greatest root development was obtained from the sand cultures.

4. In general, those cultures having the greatest transpiration for the entire growth period also showed the greatest dry weight of tops and of roots.

5. The reaction of the medium in which the plants were grown changed from an average acidity of pH 3.75 before growing the wheat cultures, to an average acidity of pH 5.94 for the water cultures, and to pH 6.66 for the sand cultures, after growing the plants for one $3\frac{1}{2}$-day period. No

correlation could be shown between the total yield of cultures and their corresponding hydrogen-ion concentration values.

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THE GREGARIOUS FLOWERING OF THE ORCHID *DENDROBIUM CRUMENATUM*

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Whenever a number of individuals of the orchid *Dendrobium crumenatum* Lindl. occur within the same general locality the plants flower simultaneously.

Such gregarious flowering is met with in other plants: for example, in certain bamboos such as *Bambusa arundinacea* (1), *Phyllostachys puberula* (2), and *Chusquea abietifolia* (3). There are, however, two striking differences between the simultaneous flowering of the orchid and that of bamboos. First, in the latter case the flowering is rhythmic. In bamboos which exhibit rhythmic sexual periodicity the time between periods of flowering, *i.e.*, the life of the plant, is about thirty-two years (3, 4). In Dendrobium the periods between flowering dates vary from a few days to several months. A glance at the dates of flowering in table 1 is sufficient to reveal the fact that there is no rhythmic periodicity here. The second striking difference between the gregarious flowering of orchids and that of bamboos is that in the latter case all the individuals of a bamboo forest are of the same age, while among an assemblage of orchids the individuals may be of quite different ages. In the case of bamboos there is accordingly good reason to regard the sexual periodicity as the expression of an innate, heritable character (3). The lack of rhythm in the flowering of the orchid and the differences in the ages of the plants which flower gregariously suggest that simultaneity here is possibly due to an external factor. The following additional observation tends further to support this belief.

Among the specimens of *Dendrobium crumenatum* in the Botanic Gardens at Buitenzorg, Java, there are plants collected from nearly all parts of the Dutch East Indies, from Riouw (near Singapore), from Sumatra, Java, Borneo, Celebes, and Ambon (a small island at the eastern end of the archipelago). These plants, shortly after being brought to Buitenzorg, all flowered on the same day, if they flowered at all; yet in their native habitats the flowering periods of the plants do not at all coincide. Thus, orchids growing in the virgin mountain forests flower on different days from those in the lowlands. Plants growing at two stations but three kilometers apart may differ in their times of flowering by one or two days. But wherever their original home and whatever the date of flowering there, the plants, when assembled in one locality, flower simultaneously with each other and with the plants which have grown in that locality from youth. Without

further investigation one would feel convinced on the basis of these facts that simultaneous flowering in *Dendrobium crumenatum* is attributable to some environmental influence.

Before considering what the determining external factor might be—the question with which this paper is chiefly concerned—it will be well to consider first how far we are justified in regarding the environment as the cause of *simultaneity* in flowering. It is conceivable that the gregarious anthesis of Dendrobium is actually the expression of the heritable disposition of the plants, and that the external environment determines only the exact *time* at which the gregarious flowering shall take place.

If we regard the plants of *Dendrobium crumenatum* which are growing in any one locality as forming their flower buds uninfluenced to any marked degree by the external environment, and as developing these buds to a definite, and in every case the same, stage of advancement, at which point growth is stopped, then we should expect that there will be at any one time many resting buds all of the same age. This being true, it is evident that, if a stimulus starts all these resting buds growing again at the same time, they will all burst into flower on the same day, assuming that the time required to cover the final lap of their development is constant in all the individuals of the species. This is what Rutgers and Went (5) believe to be the explanation of simultaneous flowering in *Dendrobium crumenatum*. The explanation is analogous to that of the "trigger type" of reaction (shock reaction) in animal behavior. The organism is "primed and cocked." The requisite external stimulus functions merely as a means of releasing the "trigger."

If this hypothesis is correct, then the environmental factor in question determines merely the *exact time* when the resting buds shall recommence growth, complete their development, and come into flower. *Simultaneity* of flowering rests upon the fact that the resting buds, which are apparently aroused to further activity by an environmental factor, are all of the same age and all require the same length of time to complete development; that is, simultaneity is due to an innate, heritable factor.

Buitenzorg is renowned for its equable climate. Tropical regions in general vary little throughout the year in temperature, but there is usually a pronounced seasonal change in moisture. No such alternation of wet and dry seasons occurs at Buitenzorg. Where, therefore, the annual precipitation is abundant and so uniformly distributed throughout the year, and where other evident environmental factors, such as temperature and light, are so constant as at Buitenzorg, it is difficult to appreciate what external stimulus might be responsible for the final development and ultimate bursting into flower of Dendrobium buds.

Burkill (6), from data obtained in the Straits Settlements, comes to the conclusion that "climatic conditions some eight days in advance of the flowering are a controlling factor" in the gregarious anthesis of *Dendrobium crumenatum*.

The data of Burkill consist in the dates of simultaneous flowering of the pigeon orchid for four years (twenty-seven flowering periods in all) and the daily rainfall figures for these years. When the two groups of data are arranged in a table, it is to be noticed that in most instances the precipitation is unusually heavy on the eighth (sometimes the seventh or ninth) day before each anthesis. Especially evident does this fact become when one considers the total rainfall for each series of days preceding all the flowering periods. The total for the eighth series of days is greater than that of any other. But among the rainfall data preceding each flowering date there are some prominent exceptions. These undoubtedly caused Burkill to conclude that

> It is not probable that the volume of the rain which falls exercises any direct influence on the flower buds; but it is quite probable that the changes in temperature accompanying heavy rainfall . . . determine the occurrence.

Wishing to ascertain if the theory of Burkill is supported by the relation between rainfall and the flowering of *Dendrobium crumenatum* at Buitenzorg, Java (where climatic conditions differ considerably from those prevailing at Singapore), I have compared the days of flowering of the orchids and the daily rainfall for twenty-seven days preceding these flowering periods at Buitenzorg. These data are arranged in table 1.[1]

If we study the table, the first evident and striking fact is that, of the precipitation totals of the various days which precede each flowering date, that of the eighth—the day on which Burkill found the total rainfall to be at the maximum—is here also the greatest. The total rainfall occurring on the eighth day previous to all the flowering dates is five ninths greater than that of the next highest. Thus, at the very outset, do we have strong support of Burkill's hypothesis. If we examine the table more critically, further favorable evidence is found.

In several instances prolonged dry periods are broken by rainfall on the eighth day before a flowering date (*e.g.*, Oct. 1, 1895; Aug. 25 and Sept. 23, 1896; and Aug. 24, 1919: a shorter dry period is broken on the eighth day preceding the flowering on Nov. 13, 1896, and on Sept. 3, 1919). It will be noticed that in each of these cases the amount of flowering was abundant.

In some instances the heavy rainfall occurred a day later or two or three days earlier. These slight divergencies can be regarded as variations of not sufficient greatness to affect materially the theory. Other exceptions exist, however, which are more disturbing. Thus, on Sept. 7, 1894, April 8, and Dec. 10, 1895, very heavy rainfall occurred on the fourteenth or fifteenth day preceding flowering. One wonders why these precipitations (and even that taking place on the twenty-seventh day preceding Oct. 17, 1894, for example) did not occasion gregarious flowering in the orchids eight days

[1] For the dates of blossoming of *Dendrobium crumenatum* I am indebted to Dr. J. J. Smith, Chief of the Buitenzorg Herbarium. For the precipitation data my thanks are due to the Meteorological Observatory of Batavia, Java.

TABLE I. *Table Correlating the Days of Flowering of the Orchid Dendrobium crumenatum with the Daily Rainfall Preceding each Flowering Date*

27	26	25	24	23	22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1		
4	—	5	2	2	—	4	1	26	10	3	27	2	16	2	—	3	—	—	21	23	4	8	13	17	32	4	II	11 January, 1894
23	50	11	—	—	2	8	18	6	17	11	6	3	21	—	5	16	—	41	6	5	17	12	1	2	9	43	II	1 March
1	11	47	20	16	2	4	38	6	6	1	4	1	3	—	2	—	—	—	14	—	3	—	18	2	47	—	III	5 April
—	15	28	24	2	10	2	—	—	13	13	7	73	13	1	4	3	—	5	25	7	—	8	16	13	3	11	II	7 May
6	40	—	25	70	—	—	1	1	6	—	—	—	10	—	28	9	16	—	7	2	—	15	—	3	—	—	II	15 June
—	—	1	—	—	6	—	—	—	3	—	2	1	86	4	—	—	16	4	5	—	—	62	30	—	3	—	II	7 September
—	2	1	86	4	—	—	16	4	5	—	—	62	30	—	3	—	—	—	14	64	152	2	—	23	—	28	III	17 "
100	6	16	38	—	36	11	—	1	—	16	2	—	—	—	—	13	5	2	13	56	—	7	55	9	78	27	V	17 October
7	55	9	78	27	4	—	2	1	—	—	7	6	27	4	28	—	—	2	15	7	6	—	—	—	32	—	I	8 November
—	—	2	15	7	6	—	—	—	32	—	—	—	6	—	7	—	—	—	38	—	12	6	5	10	2	78	II	24 "
78	9	25	18	—	46	15	5	24	6	9	62	—	6	24	28	—	—	5	10	4	21	38	36	19	18	26	IV	20 December
4	2	1	—	—	5	8	28	2	—	1	—	—	42	13	3	—	—	6	36	—	4	8	22	—	—	7	IV	1 March, 1895
5	10	11	29	5	8	5	30	9	13	19	9	111	6	9	17	11	9	2	36	12	—	—	7	4	12	29	II	8 April
33	2	6	55	—	114	4	—	—	2	27	—	—	—	—	21	—	—	14	32	4	—	66	10	2	—	—	IV	20 May
—	—	—	—	21	—	—	14	32	4	—	66	10	2	—	—	—	—	—	67	9	8	13	4	5	15	53	III	31 "
5	22	—	—	—	32	—	—	—	—	—	—	—	—	3	—	3	24	42	—	—	—	25	6	—	9	—	I	2 July
—	—	—	—	—	54	—	—	—	—	—	—	—	—	—	—	—	—	—	59	—	—	—	—	6	—	—	IV	1 October
—	—	—	4	43	—	—	—	91	3	—	—	—	4	6	—	75	10	12	40	—	23	—	7	—	43	29	III	1 November
—	5	13	35	—	—	—	—	31	65	48	25	58	70	24	11	7	10	33	2	41	29	1	4	—	17	—	III	10 December
1	—	—	—	—	5	19	—	12	41	35	22	21	—	21	12	—	44	—	48	25	—	4	—	2	25	47	IV	6 February, 1896
25	47	18	19	31	12	8	9	5	28	6	17	45	34	5	5	20	1	—	8	3	—	43	—	—	10	16	III	2 March
7	—	6	—	1	45	4	—	12	6	6	3	—	12	2	4	1	3	42	1	—	12	3	12	5	76	—	III	10 April
3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	49	3	—	11	—	4	—	1	—	—	—	IV	19 July
—	—	28	3	5	—	—	—	18	—	30	37	—	—	—	—	—	—	—	7	—	—	—	—	—	—	—	IV	25 August
—	—	3	—	6	—	5	—	—	—	—	—	—	—	—	—	—	—	—	30	17	—	—	19	65	—	—	III	23 September
2	4	—	—	51	14	—	46	1	13	—	28	30	1	—	—	—	—	—	87	18	—	2	1	2	—	14	VI	13 November
—	—	—	—	—	—	—	—	—	5	4	—	—	—	2	2	—	—	—	32	43	11	5	2	—	—	—	IV	24 August, 1919
—	—	—	—	—	—	—	5	4	—	—	—	2	2	—	—	—	32	43	11	5	2	—	—	—	—	—	IV	26 August
4	—	—	—	2	2	—	—	—	32	43	11	5	2	—	—	—	—	—	27	1	11	—	—	—	14	—	IV	3 September
308	280	231	451	293	403	97	213	286	310	272	335	430	393	120	181	210	173	253	702	346	319	328	269	189	445	412		

In the last column is given the date (day, month, and year) of flowering.

In the second column from the right are Roman numerals indicating the amount of flowering, as follows: I, very slight; II, slight; III, moderate; IV, abundant; V, very abundant; VI, profuse.

In the other columns, which are numbered consecutively from 1 to 27, is given the amount of rainfall in millimeters for each day, from the first to the twenty-seventh, preceding the day of flowering (the number of days chosen being arbitrary but of a sufficient number to give a wide range of observation).

The total rainfall for all the days which precede by the same length of time each of the flowering dates is given at the bottom of each column headed respectively 1 to 27.

later. Two possible explanations of the exceptions can be given. Either rainfall is not the exciting stimulus, or the plants can not be aroused by heavy precipitation *whenever* it occurs.

If the hypothesis of Rutgers and Went is correct, namely, that bud development to a definite point, the same in all individuals, determines simultaneity of flowering, then those exceptions in which no flowering occurs eight days after a heavy rainfall are explainable on the basis that there were no buds awaiting further development at that time. Perpetual flowering is hardly to be expected. Those other but fewer exceptions in which flowering does occur but without heavy rainfall on the eighth preceding day are explainable only on the assumption that rainfall is not the determining factor. Possibly we are forced to come to this conclusion. But it seems very likely that, whatever the stimulus is, it commonly occurs with heavy precipitation. Burkill believes that changes in temperature probably determine the time of occurrence of flowering. Since changes in temperature usually accompany heavy precipitation and may take place without rainfall, it is quite possible that temperature is the exciting stimulus. As to what the factor actually is we are quite ignorant.

The theory that the *time* of simultaneous flowering of *Dendrobium crumenatum* is determined by a climatic factor commonly associated with heavy rainfall and occurring eight days in advance of the day of flowering is supported by the Buitenzorg data so far presented. Most of the exceptions considered can be more or less satisfactorily brought into general agreement with the theory. There is, however, another and possibly insurmountable objection to the theory.

When two or more successive flowering days occur very close together, it is difficult to conceive of one (the second) lot of buds responding to the requisite stimulus eight days in advance of the flowering date and failing to respond to an identical stimulus which only two days earlier started the first lot of buds on the final stage of their development. A striking example of this was pointed out to me by Dr. Smith of the Buitenzorg Herbarium, and is recorded in the table under the dates of Aug. 24 and 26, 1919.

Considered separately, these two flowering dates fit into the theory perfectly. In both cases the day of flowering was preceded by a heavy rainfall on the eighth (or ninth) day previous to anthesis, and this heavy precipitation ended a long dry period. But when the two flowering days are regarded together, one wonders if the physiological state of the buds is quite so nicely adjusted as to leave some of them unresponsive on Aug. 24th to the same kind of stimulus which two days later, on Aug. 26th, arouses them to further development.

Such a condition is not to be regarded as impossible. In this connection it is worth noting that, while the same plant may produce blossoms on two successive flowering dates, no one shoot of any plant bears blossoms on both of two successive flowering days; that is, if a shoot of a plant

responds to the first stimulus and produces blossoms on the first flowering day, it does not respond to the second stimulus and is bare of blossoms on the second flowering day when other shoots of the same plant are in flower.

Whether or not we are near an accurate knowledge of the precise mechanism involved, it seems safe to conclude: first, that *simultaneity* in the flowering of the orchid *Dendrobium crumenatum* is the expression of an innate, heritable factor; and second, that the exact *time* at which this simultaneous flowering shall take place is determined by an external environmental factor occurring eight days in advance of the flowering date. The precise nature of this environmental stimulus is not irrefutably established. If it is not heavy rainfall it is certainly a factor commonly associated with rainfall.

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AUSTRALASIAN BOTANICAL NOTES I. QUEENSLAND AND NEW SOUTH WALES

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The botanist coming to Australia from Europe or America is at once impressed by the almost complete absence from the native flora of any plants that seem at all familiar. If he is acquainted with the Mediterranean regions or California, he recognizes the eucalypts and Acacias, so commonly planted in those regions, and perhaps he may know a few other characteristic Australian plants that have been introduced into the warmer parts of Europe and America, such, for example, as Leptospermum, Melaleuca, Callistemon, Grevillea, and others; but all of these belong to families either quite absent from the northern hemisphere, or very scantily represented there. It is evident that the vegetation of subtropical and temperate Australia has very little in common with corresponding latitudes in the northern hemisphere, and that it indicates a very ancient separation of these two regions.

The relations of the principal land masses in the southern hemisphere within the temperate zone are very different indeed from the corresponding latitudes at the north. Instead of the practically continuous land areas of the northern Eurasian and American continents, there are the relatively small and widely separated areas of Australasia, South America, and South Africa. Not only are these entirely separated from each other, but a broad belt of open ocean lies between them and the Antarctic continent, instead of there being a direct connection with the polar regions such as obtains in the northern hemisphere. As none of these land masses extends into really Antarctic latitudes, and as they are largely surrounded by water, only in the Antarctic continent itself are there to be found regions of severe frost, except at high elevations. In consequence of the prevailing temperate climate, mild winters are the rule, and evergreen vegetation prevails for the most part. Very rarely is the deciduous habit developed, and where this occurs it is due, not to cold, but to drought.

Of the three principal south-temperate regions, that of Australia is much more isolated than that of either South America or South Africa, this being especially true of Western Australia, where the peculiarly Australian vegetation reaches its culmination; and this region shows, perhaps, the highest degree of endemism known anywhere. As might be expected, the south-temperate zone has a less uniform flora than that of the north; but

nevertheless there is sufficient resemblance throughout to indicate former land connections between the different regions in earlier geologic time.

Australia comprises an area slightly greater than that of the United States exclusive of Alaska. Compared to the United States, however, it shows much less variety in its topography.

The southernmost point of Australia proper scarcely reaches the fortieth parallel, and Tasmania is only a little south of this. Hence the southernmost districts have a warm temperate climate, while more than a third of the continent lies to the north of the tropic of Capricorn, and has a tropical climate. The climate, as a whole, has a distinctly continental character, with great range of temperature, especially in the extensive arid central regions.

The principal mountains are in the extreme eastern part of the country, in some places actually on the coast. This eastern mountain region, the eastern highlands, has an abundant rainfall on the coastal side, and it is in this region that the most luxuriant vegetation is encountered. This mountainous country extends from Cape York, the extreme northern part of Australia, along the eastern borders of Queensland and New South Wales, and ends in Victoria. The highest mountain is Mt. Kosciusko, near the borders of New South Wales and Victoria. This is only a little over 7000 feet in height, and next to this are the Bellenden-Ker mountains of northeast Queensland, rising to something over 5000 feet elevation. The rainiest region of Australia is in the immediate vicinity of the Bellenden-Ker range, some stations having an annual rainfall of upwards of 150 inches. The writer visited one of these—Babinda, in August, 1921, where there was a practical demonstration that this is really a rainy district. Already over 200 inches had fallen since the first of the year, and it is safe to say that this was materially added to during his stay of three days. The precipitation falls off rapidly inland, and a large part of the interior receives less than ten inches annually.

While the rainfall is perhaps the most important element in determining the character of the vegetation, the nature of the soil also plays an important rôle. This is especially marked in the tropical and subtropical forests of the eastern coastal districts, where the tropical rain-forest types are mainly restricted to the rich basaltic or alluvial soils, while the Eucalyptus forest is almost confined to sandy soils.

A second region of relatively heavy rainfall is met with in the extreme southwest, where there is a heavy forest growth, but almost exclusively of Eucalyptus.

The plants of Australia fall into three distinct categories. The first of these includes a large number of species, mainly restricted to the coastal areas of Queensland and New South Wales, which are either identical with, or closely related to, Indo-Malayan species. The second, and much the largest, group is made up of the peculiar types which are strictly Australian

in origin. These occur in all parts of the continent, and in many regions, especially in the dryer areas, constitute the entire indigenous flora. Finally, there is a small infusion of temperate genera, such as Viola and Ranunculus, and several genera which are identical with those of the colder regions of Chile and Patagonia. This latter "Fuegian" element is best developed in Tasmania, but also forms an alpine flora in the highest mountains of New South Wales and Queensland.

An enumeration in 1914 [1] of the vascular plants of Australia gave a total number of 10,673 species, which presumably has been considerably added to since that time. According to Mueller, over eighty percent of the species are endemic.

Queensland

Soon after landing in Sydney, July 26, I proceeded by train to Brisbane, the capital of Queensland, whence I continued my journey by steamer to Cairns, in northern Queensland. This little town, in south latitude 17°, was the northernmost point reached.

The conditions in Cairns are genuinely tropical. Being winter, it was not uncomfortably hot—indeed, after the chilly winds in Sydney and Brisbane, the warm sun was very comforting.

Sailing north from Brisbane, the steamer is within the Great Barrier reef, but this was too far away to be visible. The northern Queensland coast is very rugged, the mountains rising in many places direct from the sea, and there are many picturesque mountainous islands fringing the coast. The southernmost islands and the coast are quite barren, or but scantily wooded, but along the wetter northern coast they are often densely covered with forest. In some places were pure stands of *Araucaria Cunninghamii* looking much like a northern pine forest.

The country immediately about Cairns is flat and sandy. Extensive mangrove formations fringe much of the shore and extend up the shallow creeks which abound along the coast. There are several species of mangroves in Queensland, some, like the common *Rhizophora mucronata*, with stilt roots, while the widespread "white mangrove," *Avicennia officinalis*, sends up myriads of slender pneumatophores from the roots buried in the mud.

About Cairns the forest is rather open, and is in part composed of Eucalyptus, and partly of Ficus and other tropical types. A Pandanus was very abundant, as also were cycads; but, as the latter were not in flower or fruit, it was not possible to tell whether they were Macrozamias or species of Cycas. A conspicuous large shrub was *Wormia alata*, with glossy leaves and large yellow flowers. Wormia is a common member of the strand floras all over Indo-Malaya. In the low ground around the swampy areas which were encountered here and there, were a good many ferns.

[1] Maiden, J. H. Australian Vegetation, p. 166. Federal Handbook for Australia. Melbourne, 1914.

Among the less common types recorded from this region are *Schizaea dichotoma* and Lygodium. A few specimens of Psilotum were seen, and on one of the pools a mass of *Azolla rubra*, which is not rare in many parts of Australia.

FIG. 1. Mangroves; Cairns, North Queensland.

In the gardens were the usual tropical shrubs—Hibiscus, Poinsettia, etc.—and some fine tree ferns and epiphytic orchids. Among the latter, a handsome yellow and brown Dendrobium (*D. undulatum*) was in full flower. This is said to be common in the neighborhood of Cairns.

To the south of Cairns lies the Bellenden-Ker range of mountains, the highest land in Queensland. Babinda, lying at the foot of this range, was

visited, but the almost incessant rain prevented much botanizing. This, as already mentioned, is the rainiest district in Australia, and the low wet forests are a veritable jungle, the trees loaded with creepers in great variety and exhibiting extensive groves of palms, comprising several species. Most abundant and beautiful was a graceful Archontophoenix, probably *A. Alexandrae*, but this was not certain. A very peculiar fan palm, *Licuala Muelleri*, was also common, and less abundant was the pretty little "walking-stick" palm, *Bacularia* sp.

Farther north, in the York Peninsula, are several Indo-Malayan genera, Caryota, Borassus, Areca, and others, which were not seen in the Cairns district. The pitcher plants, Nepenthes, are also apparently confined to the mountains of York Peninsula. The development of lianas was very striking everywhere about Babinda. Among the most notable were rattan palms (Calamus) of several species, several climbing Araceae (*Pothos longipes*, *P. Brownii*, and *Rhapidophora australasica*), *Piper Mestoni*, a species with showy scarlet fruits, and several which were not determined.

Epiphytic ferns, including the conspicuous *Asplenium Nidus* and species of Polypodium, were abundant, as well as other epiphytic types, like Peperomia.

In the cleared ground were thickets of two species of Rubus, and among the various other plants of the open was a rather showy Melastoma (*M. Malabathicum*).

Ferns, liverworts, and mosses were not especially conspicuous about Babinda, but along the banks of the streams were fairly abundant. A few small Hymenophyllaceae were noted, and some magnificent specimens of the giant fern, *Angiopteris* sp.

To the west of Cairns the land rises rapidly to a table land, where there is developed perhaps the finest forest in Australia. This forest contains a good many very valuable timber trees, and is rapidly disappearing before the onslaughts of the lumberman. Some fragments still survive near Kuranda, at about 1000 feet elevation, but one has to go much farther inland to find any considerable stands of untouched timber.

The railway from Cairns to Kuranda follows the gorge of the Barron River, which at the head of the gorge forms a fine cataract, the largest in Australia. Below the falls the steep walls of the canyon are heavily wooded, and among the trees can generally be distinguished, here and there, the massive cylindrical trunk and wide-spreading crown of the Kauri pine (*Agathis Palmerstoni*), perhaps the most prized of all the timber trees of this region. Near the brink of the falls, a small piece of forest has been reserved in which are standing two or three fine specimens of this tree, the largest with a girth of about 16 feet.

The forest about Kuranda was somewhat more open than the lowland rain forest, and the trees were taller, often with straight boles of great height. Only one Eucalyptus, *E. tesselaris*, was seen in this district, the

forest being almost exclusively of the Malayan rain-forest type. Lianas in great variety were noted, perhaps the commonest being species of Vitis, but climbing Araceae like those seen at Babinda were conspicuous, and the

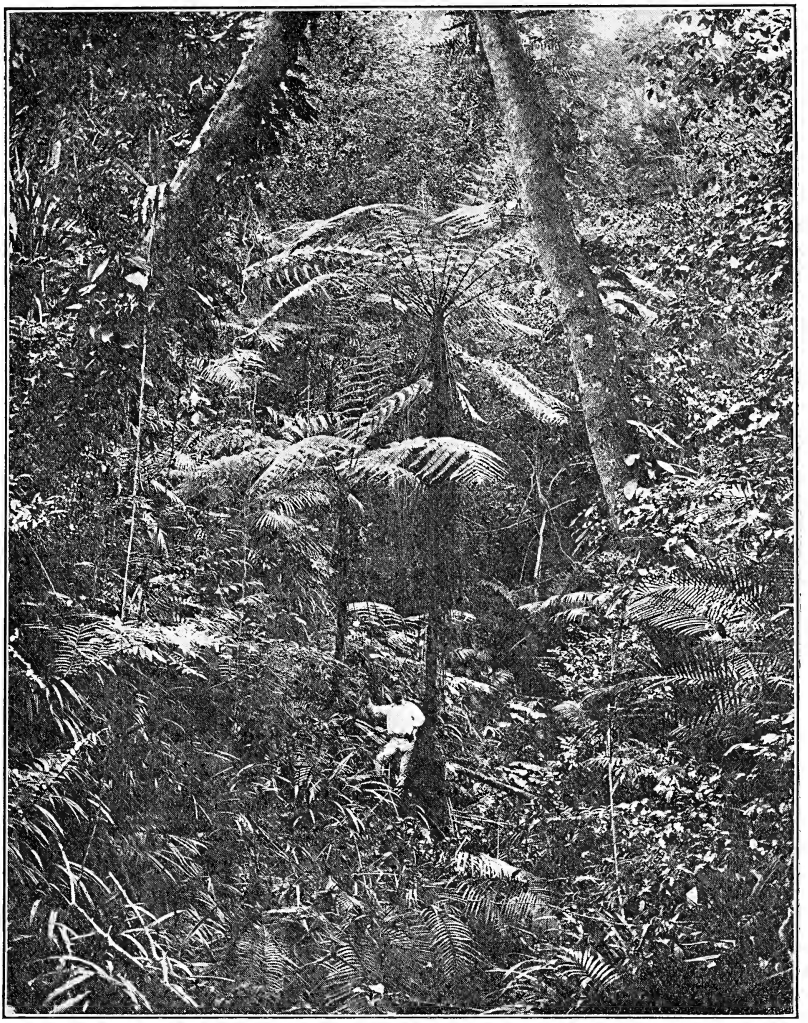

FIG. 2. Rain forest, North Queensland; in foreground and at the right, *Calamus* sp.; in the middle, *Alsophila australis;* on the tree trunk at left, *Asplenium Nidus* and *Pothos* sp.

rattan palms (*Calamus* sp.) made impassable tangles, especially at the edge of the forest. No barbed-wire entanglement could be more impenetrable than a rattan thicket, as one soon learns to one's sorrow. Another dreaded pest of these forests is the tree nettle, *Laportea moroides*, a rank-growing weed some ten or fifteen feet high, whose touch is torture. In southern

Queensland is a second species, *L. gigas*, which is a tree of large size. Among the trees which may be called truly Australian were species of Grevillea and Casuarina growing mostly in the more open districts. A Pandanus was also common, and a true Cycas, *C. media*. This genus is found in Australia only in tropical Queensland, and the same is true of the exclusively Australian genus Bowenia. The latter, with its bipinnate leaf, suggests a fern like Pteridium, rather than a cycad. *B. spectabilis* is not uncommon about Babinda.

Ferns are rather more in evidence than in the lower country. Two species of tree ferns were noted, *Alsophila australis* and *A. Rebeccae*. A few filmy ferns were seen, but these were not common. Other ferns noted were *Marattia fraxinea* and several species of Adiantum; *Lygodium scandens* was very common, and *Gleichenia linearis*, *Asplenium Nidus*, and other epiphytic species were frequent.

Through the kindness of Mr. A. H. Belson, of Jungaburra, I had an opportunity of visiting a tract of untouched timber, which gave an excellent idea of the character of the forest of the higher table-land. Jungaburra lies at about 2500 feet elevation, and although it is in latitude 17° or thereabouts, its winter climate is far from tropical. Evidences of severe frost, sufficient to cut back bananas and other tender plants, were to be seen in many places, while at Herberton, at a somewhat higher elevation, fourteen degrees of frost were reported.

The forest near Jungaburra was more open than that at Kuranda, and the lianas and epiphytes were rather less in evidence. Many of the trees are of great size, with tall, straight trunks, often supported by buttresses, so common in the tropical rain forest. Among the trees of this forest, probably the majority were species of Flindersia, a genus usually placed in the Meliaceae but referred by Engler to the Rutaceae. Some of these are known locally as "ash," "beech," "maple," "hickory," from some supposed resemblance of the wood to that of these very different trees.

Formerly abundant, but now becoming very scarce, is the "red cedar," *Cedrela toona*, which reaches a gigantic size, sometimes ten feet or more in diameter. Other abundant species noted were *Xanthostemon pubescens*, of the Myrtaceae, *Cryptocarya Palmerstoni*, "black walnut," and *Tarrietia Argyrodendron*, "crow's-foot elm." A few fine specimens of *Agathis Palmerstoni* and *Podocarpus elata* were seen, but these species evidently were not abundant.

In the Queensland "scrubs," the local name for the rain forest, there are several Proteaceae which attain the size of large trees and yield valuable timber known in the trade as oak. The most familiar of these is the "silky oak," *Grevillea robusta*, often grown in California as an ornamental tree; but several other genera occur, *viz.*, Embothrium, Stenocarpus, Carnarvonia, and Darlingia. The two latter are monotypic.

As in most tropical rain forests, the genus Ficus is conspicuous, and in

the neighborhood of Jungaburra are some trees of gigantic size (fig. 3). One, with a compact trunk of closely interlaced roots, was said to have a girth of 120 feet, and the great crown of foliage was in proportion.

FIG. 3. Giant Ficus; Jungaburra, North Queensland.

From Cairns I returned by steamer to Brisbane, the chief city of Queensland, nearly a thousand miles south. The botanical garden in Brisbane is not very large, but, as it was established many years ago, it contains many fine old trees and shrubs. The climate of Brisbane is subtropical, and there is a pretty large collection of palms and other tropical types, as well as those of more temperate climes. A small grove of giant bamboos, sheltering palms, and tree ferns was perhaps the most striking feature of the garden. Of the flowering shrubs, a fine lot of Indian Azaleas may be mentioned. These do particularly well in the coastal region of Australia, and were especially magnificent in the gardens in Sydney, on my return from the north.

The immediate vicinity of Brisbane offers little of botanical interest, but through the kindness of Mr. C. T. White, the government botanist, I was able to make two trips into the country, and thus had an opportunity to see something of the flora of South Queensland.

The first expedition was only a short distance from Brisbane, and was especially interesting, as it illustrated most beautifully the sharp line

separating the Eucalyptus forest from the "scrub" or rain forest. This seems to be due mainly to soil conditions, the rain forest being confined to the richer basaltic or alluvial soils. Some of the eucalypts of this region are very tall and beautiful trees, and valuable for timber. The following species were noted: *E. tereticornis*, *E. paniculata*, *E. propinqua*, *E. maculata*, and *E. tesselaris*. The nearly related *Angophora subvelutina* was also seen, and several of the characteristic Acacias—*e.g.*, *A. Cunninghamii* and *A. Maidenii*.

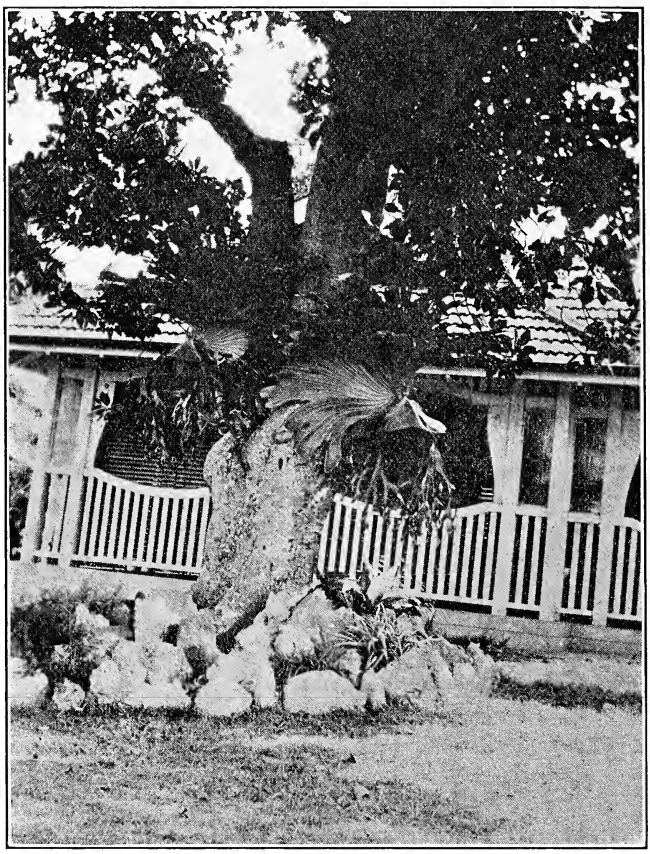

FIG. 4. *Platycerium grande.* Botanical Garden, Brisbane.

In the scrub, which included some eucalypts also, the most notable tree was *Araucaria Cunninghamii*. Other trees and shrubs noted were several species of Flindersia, *Carissa ovata*, *Canthium buxifolium*, *Pivetta indica*, *Pseudomorus Brunoniana*, *Melaleuca* sp., *Vitex Lignumvitae*, *Ratonia tenax*, *Sideroxylon myrsinoides*, and several others.

The second excursion in South Queensland was to the Blackall Range, a mountainous district about 75 miles north of Brisbane. The railway to

the north is nowhere far from the coast and passes in places through patches of jungle of a decidedly tropical aspect. In the low ground, extensive groves of beautiful palms (*Archontophoenix Cunninghamii*) were seen, especially in the more northern districts.

After leaving the main line, we proceeded by a primitive tram line to Mapleton, at an elevation of about 2000 feet; but even at this height, palms were seen.

Most of the forest in the immediate neighborhood had been cut, but there were still remnants which showed what it had been. The forest here was of two sorts: "scrub," consisting almost entirely of true rain-forest types; and a mixed forest containing magnificent specimens of eucalypts (*E. microcorys*, *E. acmenioides*, and *E. pilularis*). With these were fine specimens of the related *Syncarpia laurina* and *Tristanea conferta*, upon whose smooth trunk was growing an epiphytic orchid, *Dendrobium acuminatum*. Of the smaller trees and shrubs, the following were the commonest: *Casuarina torulosa*, *Rhodamnia trinervia*, *Schizomeria ovata*, *Trochocarpa laurina*, and *Eupomatia laurina*. Some attractive flowers were seen, but nowhere in Queensland were the flowers as abundant or as showy as in parts of New South Wales and especially in Western Australia. Several showy Papilionaceae may be mentioned, species of Hovea, Glycine, Platylobium, and Kennedya, and an exceedingly pretty ground orchid, *Caladenia carnea*, was not uncommon. Hibbertias (Dilleniaceae) with showy yellow flowers were fairly abundant, and a true violet, *V. hederacea*, was about the only representative of the boreal flora.

Several species of Loranthus were seen, some with showy red and yellow flowers. There are many Australian species, and it is one of the most characteristic features of the Australian flora. Ferns were not especially abundant, but several species were common. Among these were *Davallia pyxidata* and *D. dubia; Pellaea paradoxa*, *Cyclophora serpens*, and *C. confluens*.

The country about Mapleton is fertile, and the climate is sufficiently tropical to permit of the successful cultivation of sugar cane, bananas, and oranges.

Not far from the village is a fine gorge into which a small cataract falls. This gorge has, up to the present, remained undisturbed, and offers an excellent example of the luxuriant rain forest which has been mostly destroyed elsewhere in the neighborhood. The steep walls of the gorge are clothed with a dense forest, in which only a few eucalypts were seen. Along the streams at the bottom of the gorge the aspect of the forest was quite tropical. Graceful palms and tree ferns fringed the streams, and there were a good many lianas, among them the southernmost representative of the rattans, *Calamus Mülleri*, which reaches well into New South Wales. On the trees were seen some interesting epiphytes, including orchids and ferns. The commonest of the orchids was a Cymbidium, not, however, in

flower. Of the epiphytic ferns, much the most striking were the two species of Platycerium, *P. grande* and *P. alcicorne*. Many young specimens of these were seen growing on rocks as well as on trees. Some aroids were seen, but these were not nearly so conspicuous as those in the scrub of North Queensland. A few specimens of the cycad *Macrozamia spiralis* were seen, but much smaller than specimens noted in New South Wales.

The most striking feature of this district was the "Bunya" pine, *Araucaria Bidwillii*, a much finer species than the more abundant *A. Cunning-*

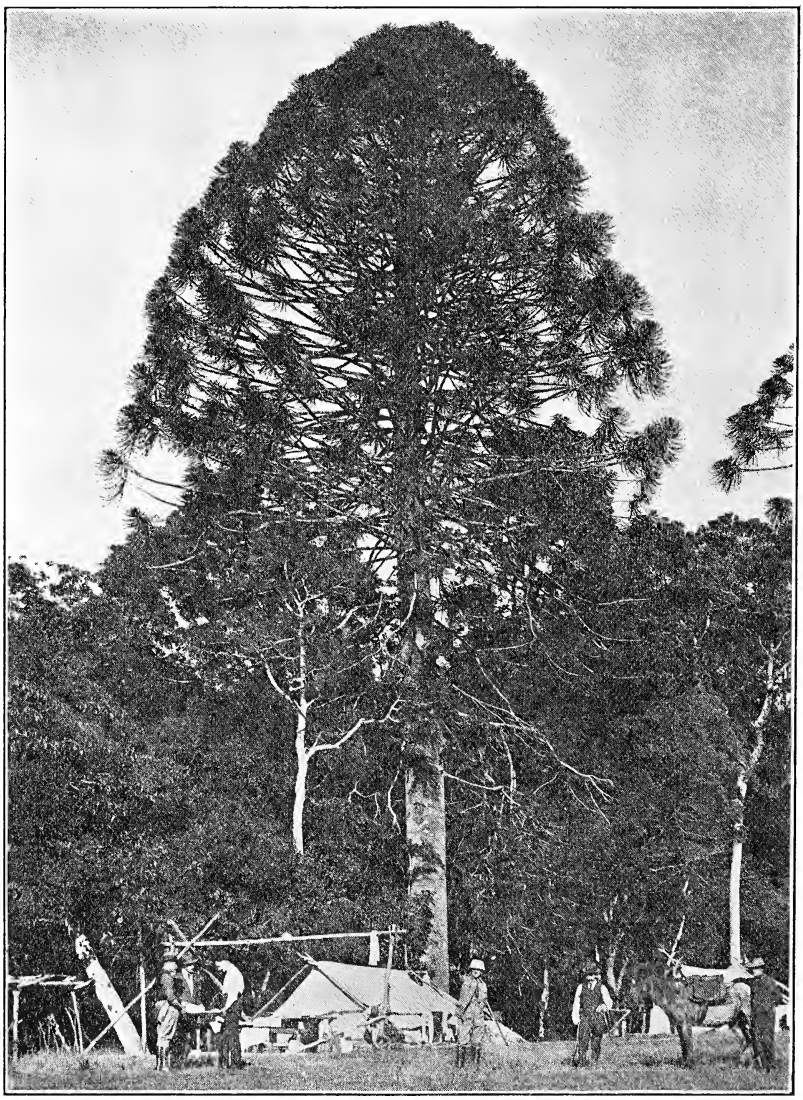

FIG. 5. *Araucaria Bidwillii;* South Queensland. Photograph by Mr. C. E. S. Fryer.

hamii. *A. Bidwillii* is common in cultivation, and fine specimens are growing in various parts of California. The tree is decidedly restricted in its range, being confined to a rather limited area in southern Queensland. The Bunya mountains have extensive forests of this species, but outside this area they occur only as scattered individuals. Some very fine specimens were seen in the Mapleton forest, their great smooth domes of foliage overtopping the other trees. As the trees grow old, the smaller twigs fall off from the base of the horizontal branches, leaving them quite bare for most of their length, and the leafy twigs form a bunch of foliage at the tip. These Araucarias were greatly prized by the aborigines for their large, edible seeds.

Before returning to Brisbane, I stayed over night at Palmwoods, a station on the main railway line adjacent to a forest with fine groves of palms. These were practically all *Archontophoenix Cunninghamii*, and in the low, swampy ground formed pure stands of considerable extent. In the higher land, they were scattered among other trees. Perhaps no plant formation in Australia is more striking to the American botanist than these beautiful groves of tall, graceful palms. Few palms rival in beauty this species, with its smooth, slender trunk and crown of feathery foliage.

Along the railway embankment, in places, were masses of *Gleichenia linearis* and *Lycopodium cernuum*, a very common association in many of the warmer parts of the world. A very pretty blue iris was noted (*Patersonia* sp.), a very common genus in New South Wales and West Australia.

The railway from Brisbane to Sydney ascends to about 2000 feet 100 miles west of Brisbane, and then follows the table land southward. This table land includes the Darling Downs, a region of deep, rich, black soil, resembling the black "adobe" of California.

The precipitation in this region is rather uncertain, but in years of good rainfall, like that of 1921, heavy yields of grain, hay, and fruits, as well as dairy products, make this one of the richest agricultural districts in Australia. The country is an open one with no heavy forest, and at the time of my visit, in early August, the luxuriant growth of young grain and alfalfa gave promise of a bountiful harvest.

The Sydney end of the railway passes through a much less promising country and is practically at sea level. The soil is largely a poor, sandy one, and outcrops of rock are seen everywhere. There is the typical Eucalyptus forest, interspersed with golden-flowered wattles (*Acacia* spp.) and the characteristic Casuarinas and Melaleucas. Here and there, clumps of cycads (Macrozamia) were noted; and in a few places, where the soil was richer and moister, specimens of the Australian fan palm, *Livistona australis*, were seen. It was rather early for the showy flowers which abound in this region, but pretty pink Boronias were recognized, as well as a handsome trailing leguminous plant, with fine blue flowers (probably Hardenbergia). Many "grass trees" (*Xanthorrhoea* sp.), another peculiar Australian type, were seen everywhere.

The heavy forest in Queensland is restricted to a relatively narrow strip adjacent to the east coast. Inland the conditions are not favorable for tree growth, and a very large portion of the 600,000 square miles of the state has no heavy forest growth. Maiden has summarized the situation as follows:

> Westerly there are broken elevated table lands with rolling country beyond, much of it covered with open forest, of which Eucalyptus is an important constituent, and then sloping away to the centre of Australia are found conditions rarely favorable to tree-life.[2]

New South Wales

As the ship enters the celebrated harbor of Sydney, one sees the numerous headlands and rocky promontories covered with the familiar Eucalyptus, interspersed with numerous characteristic low trees and shrubs, Casuarina, Melaleuca, Leptospermum, Callitris, and many others. The great city has spread of late years over most of the hills surrounding the harbor, and the native vegetation is rapidly disappearing, although in a few places there are parks or other reservations where it is being protected.

A walk over such areas as are still intact is full of interest. In the spring the sandy soil between the trees and larger shrubs produces many charming flowers—most of which are quite unfamiliar to the newcomer. On the rocky banks, especially where water seeps through, there are some very interesting ferns and liverworts, and sometimes huge sundews, giants of their kind. Some of the large shrubs at this time are also extremely ornamental: Grevilleas with pink or scarlet flowers; Acacias, masses of golden bloom; Melaleuca and Leptospermum, loaded with exquisite white flowers.

Approaching the wharf in Sydney, one catches a glimpse of the beautiful botanical gardens which extend down to the water. Adjoining the gardens, on a conspicuous elevation, is the picturesque Government House surrounded by stately Norfolk Island pines.

The botanical gardens in Sydney are extremely interesting botanically, as well as being very attractively laid out. The climate of Sydney is warm enough to permit the growth of all subtropical, and even of some tropical, plants. The garden is handicapped by a rather poor, sandy soil, and suffers at times from lack of water during the long, hot summer. This, however, was not evident in the spring, when an abundance of rain made everything look very fresh and attractive. Surrounding the garden on two sides is the "Outer Domain," a public park devoted to playgrounds and other similar purposes. A notable feature of this domain is an avenue of giant banyans, *Ficus macrophylla*, a native species known as Moreton Bay fig. On one side of the domain English oaks were planted, and had reached a very respectable size.

[2] Maiden, J. H. Australian Vegetation, p. 207. Federal Handbook for Australia. Melbourne, 1914.

In July, corresponding to our January, there was not very much in the way of floral display in the gardens. Poinsettias were in bloom, but looked rather pinched, not enjoying the cold westerly winds which then prevailed. Several specimens of an Erythrina (probably *E. indica*) were pretty well in bloom, their vermilion flower clusters being most conspicuous on the quite leafless branches.

In September, when the gardens were visited again, a great difference was noted. The spring display of flowers was about at its best, and included a great variety of bulbs and herbaceous bedding plants as well as many flowering trees and shrubs. The most beautiful feature of the gardens at this time was the magnificent display of Indian Azaleas, which seem to find the Sydney climate exactly suited to their needs. Huge bushes ten feet high or more were solid masses of gorgeous bloom, white, pink, and crimson.

Probably the collection of palms will first attract the attention of the botanist. These comprise practically all of the warm-temperate and subtropical species, but also include a considerable number of truly tropical genera, such as Oreodoxa and Caryota.

Another notable feature is the remarkably complete collection of cycads, nearly all the known genera being represented. Of the conifers, the fine old specimens of *Araucaria excelsa* take first place. One of these was planted in 1818 and is now over a hundred feet high.

Screw-pines, tree ferns, and a great variety of Australian and exotic trees and shrubs, as well as the more familiar things like Magnolias, Rhododendrons, flowering peaches, and crab apples, combined to produce most beautiful effects.

Still later, in November, another set of plants was in bloom; roses and various familiar herbaceous perennials were in full flower, and tropical-looking Bignonias and the blue Jacaranda made a gorgeous show.

The collection of succulents is an excellent one. American Cacti and Agaves, and the South African Aloes and Euphorbias, were equally at home. On some of the rockeries were great masses of a very fine native orchid, *Dendrobium speciosum*, with long racemes of handsome lemon-yellow flowers.

It was a great pleasure to meet again the director, Mr. J. H. Maiden, F. R. S., to whom the writer is indebted for many kindnesses.

The country about Sydney is rich in showy flowers, which are seen in profusion on the street flower-stands. Especial favorites are species of Boronia and the gorgeous "waratah" (*Telopea speciosissima*).

A visit was made to a point about 25 miles north, in a region which was still quite undisturbed. This was at an elevation of about 600 feet and was the usual open Eucalyptus formation with lower trees and shrubs interspersed, among which grew a profusion of beautiful low flowering shrubs and a small number of herbaceous plants including two or three orchids.

Pink-flowered Boronias and Eriostemons, belonging to the Rutaceae, were very abundant and beautiful; the Proteaceae were represented by

Banksias, some of which were small trees, and the lower-growing species of Grevillea, some with pretty pink flowers; Hakea, Persoonia, and Isopogon; *Lambertia formosa*, with brilliant scarlet flowers, was perhaps the finest of the Proteaceae. Yellow-flowered Hibbertias were very common, and other distinctly Australian types were Tetratheca (Tremandraceae), Dampiera (Goodeniaceae), and Comesperma, a very beautiful blue twiner belonging to the Polygalaceae. Various Papilionaceae, mostly species with showy yellow or red and yellow flowers, were very abundant, and played an important rôle in the gorgeous flower show.

Another most interesting trip was made by motor with Professor A. A. Lawson, of the University of Sydney, an old associate of the writer, to the National Park, one of the finest pieces of scenery in Australia. Much of the country between Sydney and the park is of the same character as that just described, and there were very beautiful displays of flowers in great variety. At one place, however, along the rocky banks of a stream, very different vegetation was encountered. On the dripping rocks were masses of liverworts and sundews, while in the sheltered nooks we found the interesting fern *Todea barbara* and the still more interesting Tmesipteris, whose life history was first made known through Professor Lawson's careful investigations.

The drive through the Bulli Pass took one back to tropical Queensland. The road was shaded by huge trees, covered with creepers, and masses of splendid tree ferns and tall palms (*Archontophoenix Cunninghamii* and *Livistona australis*), gave a most tropical appearance to the landscape.

The coast here is very picturesque, with sheer cliffs falling to the more level land at the shore, and from the top of the cliffs one can see the bold shore line with headland behind headland, in both directions, and the curved sweep of long beaches at their feet.

The return to Sydney through the National Park followed for several miles the gorge of a stream, and the luxuriant forest vegetation was much like that of the Bulli Pass. The forest was a mixed one; some of the eucalypts (probably *E. pilularis*) were enormous, and the closely related *Angophora* sp. was not uncommon. *Eugenia* sp. and some others were of the true rain-forest type. Tree ferns and fan palms were abundant.

Of the flowers noted on this excursion, two stand out preëminently. One was a mass of the giant torch lily, *Doryanthes excelsa*, a plant related to our Agaves. From the cluster of broad leaves, five or six feet high, rises a stout scape, ten or fifteen feet high, bearing at the top a huge cluster of great scarlet lily-like flowers, surrounded by large red bracts. The other was a colony of the beautiful orchid, *Dendrobium speciosum*, growing on a rock ledge, and in fullest bloom.

One of the most interesting districts, botanically, in New South Wales is that of the Blue Mountains to the west of Sydney. The Blue Mountains form the edge of the elevated plateau which slopes westward to the plains

of the interior. The highest point is about 4000 feet above sea level, and in places there are deep and abrupt gorges cut in the sandstone rocks; and these gorges, which are well watered, support a rich and beautiful flora and provide fine collecting ground for the botanist.

In company with Dr. Lawson, I spent a couple of days at Wentworth Falls, in the immediate vicinity of one of the finest of the gorges. The plateau is covered with the usual open Eucalyptus forest with the accompanying vegetation like that of the lower country; but, as Wentworth Falls is over 2000 feet above Sydney, many plants at this time (September 10) were not yet in full flower.

Just above the gorge is a somewhat dry table land which has rather the aspect of an open moorland. The Eucalyptus trees were small and scattered, and most of the ground was covered with a thin chaparral-like scrub made up principally of Proteaceae of various kinds—Banksia, Hakea, Grevillea—and Leguminosae, including such showy genera as Pultenaea, with fine yellow flowers, blue Hardenbergias, scarlet Kennedyas, dwarf Acacias, and many others. Epacridaceae were also abundant, comprising species of Epacris, Styphelia, and Leucopogon, while several species of Leptospermum and a Melaleuca were the commonest of the Myrtaceae. Boronias were abundant, and the somewhat similar Tetratheca displayed its pretty pink flower in many places. A curious little violaceous flower, *Ionidium filiforme*, was seen for the first time, and the brilliant blue *Lobelia gibbosa* was noticed in a few places, while a Campanula-like Wahlenbergia was not uncommon. In the damper spots, little Droseras were found, but these were much better developed on the dripping rocks in the gorge. A very pretty little orchid (*Caladenia* sp.) was seen, and an attractive liliaceous plant, *Thysanotus tuberosus*, with lavender-fringed petals, was common.

A few specimens of *Callitris Muelleri* were the only conifers growing on the table land, but in the gorge, near one of the waterfalls, were seen several specimens of the extremely rare *Pherosphaera Fitzgeraldi*, small bushes looking something like a dwarf juniper. Several species of Casuarina are found in the neighborhood of Wentworth Falls, and one, *C. nana*, which was very common on the table land, was only a foot or so in height.

An interesting but rare fern of the table land was *Schizaea bifida*, of which a few specimens were seen, growing in the barren, sandy soil.

The descent to the bottom of the gorge was full of interest. Well-made trails lead in various directions, and, as the gorge is a state reserve, the vegetation has been preserved intact and furnishes the botanist an unspoiled sample of the flora of the region.

The dryer slopes along the paths show much the same vegetation as the plateau above; but the sheltered and well-watered ravines and gullies exhibit a fairly tropical profusion of plant life. In many places water oozes out between the rock strata, and the dripping banks and cliffs harbor a wealth of curious and beautiful plants.

Proteaceae are extremely abundant in this region, over thirty species being recorded. First in size in the dryer soils were some half-dozen species of Banksia, the largest, *B. serrata*, being a very characteristic small tree, with thick, rough, corky bark and conspicuously serrated leaves. Few fresh flowers were seen, but the remains of the last year's inflorescences were conspicuous. The flowers are in dense oblong heads and are known locally as "honeysuckle." Several Grevilleas were common, and, in addition to the species observed in the open country above, there were other species as well as species of Lomatia, Xylomelum, Petrophila, and Isopogon. Several species of Persoonia were common, their glossy, bright-green leaves differing much from the foliage of most Proteaceae. The waratah (*Telopea speciosissima*) was not uncommon but not yet in full flower. About Sydney it was in full bloom, and the gorgeous blood-red inflorescence, surrounded by big scarlet bracts, make it one of the most magnificent of the many beautiful Australian flowers.

The characteristic Leguminosae and Myrtaceae, Casuarinas, and other shrubs were associated with the Proteaceae, and also several forms not seen at the top. Two Cunoniaceae were noted, *viz.*, *Bauera rubioides*, a prostrate shrub with pink flowers, and *Callicoma serratifolia*, a tall shrub with large, serrate leaves.

The dripping rock walls supported a characteristic flora. In a mass of oozy material, partly made up of algae, were some most interesting liverworts, mosses, and ferns, as well as a good many flowering plants. Among the latter were some particularly beautiful heaths (*Epacris* sp.) with pink and white flowers, and several species of sundews abounded in these miniature bogs. One of these (probably *Drosera binata*) had leaves with petioles a foot or more in length and the forking laminae as broad as one's hand, divided into narrow segments covered with the characteristic tentacles. In contrast to this giant species were tiny flat, cushion forms more like those of northern bogs.

As elsewhere in Australia, herbaceous plants are not very much in evidence, and only a few of them, like species of Ranunculus and Viola, are familiar to the northern botanist. Two characteristic orchids were noted, one a species of Pterostylis with curious greenish flowers, known popularly as "green-hoods," the other a small epiphytic Dendrobium which, however, was not in flower.

The bottom of the gorges supports a fine forest, of somewhat the same type as that seen in the National Park. Magnificent eucalypts, with tall trunks and lofty crowns, were associated with the nearly related genera Angophora and Syncarpia, and the fine *Tristania nereifolia*. Two species of Eugenia are also found in this forest, as well as *Stenocarpus sinuatus*, belonging to the Proteaceae. *Pittosporum undulatum* also occurs, and *Pomaderris elliptica* (Rhamnaceae).

The forest in these moist gorges is very similar to that in southern Queensland, and approximates the mixed rain forest of that region.

The Blue Mountains have an extensive and varied pteridophytic flora. Over forty species occur in the immediate vicinity of Wentworth Falls, and comprise a remarkable number of especially interesting species. On the wet rock slopes, and in the crevices, were two species of Lycopodium, *L. laterale* and *L. densum*, whose gametophytes can usually be found with a little searching. Two species of Selaginella, *S. uliginosa* and *S. Preissiana*, were abundant. *Tmesipteris tannensis*, whose interesting life history has recently been revealed, was found in a few places in the gorges, where Professor Lawson had frequently collected it. The ferns were very abundant, especially in the gullies and on the wet, rocky banks, but there were also a considerable number of more or less xerophilous species, like *Schizaea bifida* already mentioned. The ubiquitous *Pteridium aquilinum* is abundant, as it is in many parts of Australia. A second Schizaea, *S. rupestris*, was found on the wet rocks in company with Lycopodium, Drosera, and other bog plants, and on the somewhat less wet rocks was an abundance of *Gleichenia* spp. Four species occur in this region, and are very common and characteristic ferns.

The finest development of ferns, however, was in the damp, shady gullies near the bottom of the gorge. These fern gullies are extraordinarily beautiful. Clear streams form series of falls and cascades, and the fern growth is very luxuriant and includes some extremely fine species. The tree ferns are represented by two species of Alsophila, *A. australis* and *A. Cooperi*, which formed extensive groves of great beauty. Their slender trunks were sometimes twenty feet or more in height, and the crowns of fronds were very luxuriant and perfect. Seen from above, this carpet of interlaced giant fronds was extraordinarily beautiful.

Next to the true tree ferns, the magnificent fern *Todea barbara* was the most conspicuous. This sometimes forms a short trunk, but can hardly rank as a true tree fern. A second species, *T. Fraseri*, is very different in habit, and is often placed in a distinct genus, Leptopteris. This, like the two closely related New Zealand species of Leptopteris, grows in very wet places. It was growing where it was constantly wet with the spray of the falls, and its thin, translucent foliage much resembles that of the Hymenophyllaceae.

The latter were represented by several small species of Hymenophyllum and Trichomanes, but these are said to be much better developed in the cooler forests of the higher elevations.

Most of the widespread genera of Polypodiaceae are well represented, *e.g.*, Pteris, Lomaria, Blechnum, Asplenium, Doodia, Polypodium, Aspidium, Davallia, and Adiantum. These were abundant in all the moister places, often completely carpeting the forest floor. There were not many epiphytic species noted, but on the whole both the fern flora and the bryophytes were much more in evidence than in most of the places visited in Queensland.

The bryophytic flora of the gorges was also extremely interesting. In

the boggy places along the trail were occasional patches of Sphagnum, and other conspicuous mosses were species of Polytrichum and Dawsonia.

Hepaticae abounded on the rocks and on the trunks of trees, and quite an extensive collection was made, which has not yet been worked up. As usual, the foliaceous species were the more common, but thallose types were abundant, especially in the shady fern gullies.

The most interesting find was *Podomitrium phyllanthus*, an Australasian species seen for the first time. This was very common, and a fine lot of material was secured. Very much like it in appearance were some of the species of Pallavicinia and Symphyogyna, which were also abundant. The Pallavicinias included species both of Eupallavicinia and Mittenia. Aneura, as is usual in such localities, was represented by a number of species, including one very large one which closely resembled *A. maxima* of the East Indies.

These gullies with their perpetual shade and moisture, and the corresponding luxuriant growth of forest trees and moisture-loving ferns and liverworts, and the barren sandy moorland on the table land a thousand feet or so above them, with its predominantly xerophytic vegetation, afford one of the most remarkable examples that has come to my notice of the differences in vegetation within a limited area due to the amount of moisure.

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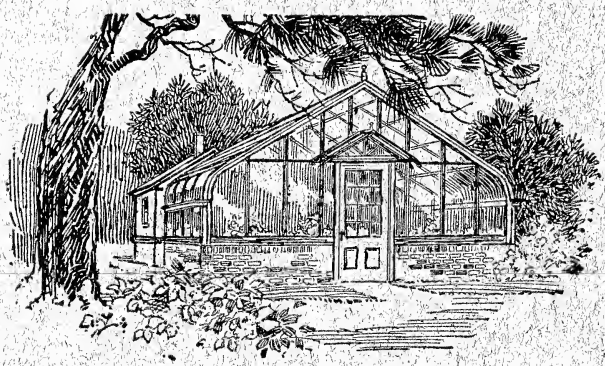

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ALTERNATION OF SEXES AND INTERMITTENT PRODUCTION OF FRUIT IN THE SPIDER FLOWER (*CLEOME SPINOSA*)[1]

A. B. STOUT

(Received for publication April 6, 1922)

Irregularities in the formation of reproductive organs, such as are seen in the phenomena of intersexualism in both plants and animals, have two points of special interest. First, they involve a particular type of sterility of various grades and degrees of expression, which in plants often affects the production of fruit and seeds and becomes a matter of practical importance in respect to crop production and in the breeding of various economic plants. A second point of interest is in the bearing which the phenomena of intersexualism have on questions of sex differentiation, the alternation of sex, and the evolutionary tendencies in reproduction.

In its general significance, several points regarding sterility from intersexualism are clear. In plants it tends to the alternative development of one or the other kind of sex organs, giving, in comparison to the fundamental condition of hermaphroditism, a one-sided sterility. There is incomplete development or abortion of one or the other of the sex organs which is discriminating and which results in alternative development, with, however, many grades in the relative development. Thus, in plants, the so-called "sterile" intersexes are, in general, individuals that are predominantly male and often highly functional as such. These individuals are sterile only in the sense that they are fruitless. Also the so-called "self-sterile" individuals and varieties of plants, as is well shown in the cultivated grapes in which sterility from intersexualism is well marked, are predominantly female and able to function feebly or not at all as males. They are productive of fruit only when properly pollinated from male or hermaphroditic individuals. Very seldom, if ever, is complete sexual impotence for a plant as a whole seen as a condition of intersexualism, as is frequently the case in sterility from hybridity.

But in many cases of intersexualism in animals, to which attention has recently been especially directed, the complete sterility of individuals is very frequent. Here, however, the condition arises in dioecious forms and involves the partial change of an organ from one sex to the other after

[1] Contribution from the New York Botanical Garden, No. 239.

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differentiation has been partly achieved. This often results in a more or less complete sexual impotence or sterilization, a condition which has naturally been very generally regarded as abnormal and pathological. This is, however, not the case in those species of plants which are prevailingly dioecious or monoecious, for here, as well shown in the muskmelons, there is a tendency to produce flowers that are fully functional as hermaphrodites.

Whether, however, intersexualism results in complete sterility, as it frequently does in dioecious animals, or in one-sided sterility, as is the rule in hermaphroditic plants, the physiological basis for these variations in sex is to be regarded as most fundamental in the determination and expression of sex.

It is, furthermore, to be recognized that the mixture of sexes, with blending and changes in the character of the organs, often results in a wide range of variation in the morphological character of the different sex organs produced by a single individual. In many plants, the flowers on a single individual may be staminate, pistillate, and hermaphroditic, with also many intergrading types, thus exhibiting many grades of sexual impotence with marked differences in the ability to produce fruit.

These cases of partial variability in sex are of special interest, for here the various conditions of alternative impotence with corresponding irregularities in the production of fruit are all seen among the flowers of a single individual. In such cases there is also opportunity to observe whether the variations are irregular and sporadic or whether they are related to a definite period in development or are otherwise periodic. It is with special reference to these questions that the changes in the character of the flowers of *Cleome spinosa* L. are here reported as decidedly alternative and repeatedly cyclic, resulting in the intermittent production of fruit.

Observations on Cleome spinosa

This species is most favorable material for a study of variation in the sex of flowers in relation to the development of the plant as a whole. It has long been known as having mixed flowers, yet the species has not become dioecious. All the individuals of the species are apparently quite alike in respect to the general range of variations in the sex of the flowers. The species is a quick-growing herbaceous annual. The first flowers open on the main raceme when the plant is relatively small—about two feet tall—and while the lateral branches are scarcely visible. The main raceme continues to elongate, producing flowers daily, often for a period of from eight to twelve weeks. Meanwhile a dozen or more lateral branches develop, and these may in turn branch. All the branches grow rapidly and produce flowers in abundance. When autumn arrives, well-grown plants are five or more feet tall with a spread of branches of as many feet in diameter. There has been a long period of bloom, often covering as many as ninety days, and this has been for the most part coincident with the period of

rapid vegetative development. By far the greater amount of the vegetative growth of the plant takes place after the blooming begins. The first fruits are ripe and shed their seeds when the plant is only about half grown. The period of vegetative vigor overlaps that of the flowering and reproductive vigor in a decided degree and to an extent seldom seen in plants. Only during the last few days of bloom do vegetative growth and vigor noticeably wane.

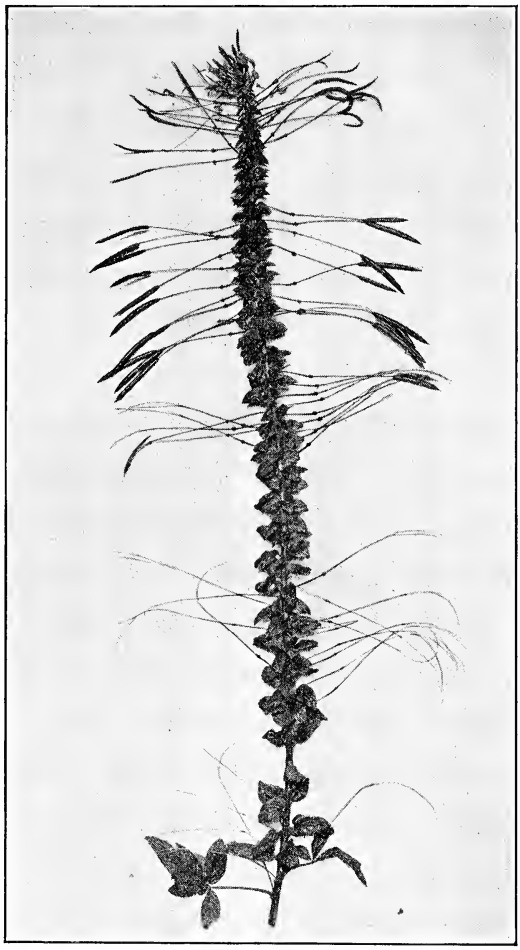

FIG. 1. Main raceme of a plant of *Cleome spinosa* at the close of the blooming period, showing the intermittent production of fruit. This raceme was about $3\frac{1}{2}$ feet long. The pods on the lower half have dehisced.

At the close of the growth of the plant, about September 15th to October 5th as grown at the New York Botanical Garden, the main branch of a plant from the point where the flowers are first produced appears as shown

in figure 1, the raceme being about three feet in length. Lateral branches are similar but frequently somewhat shorter. It is to be noted that the pods are in groups separated by sections of the stem upon which no fruit was formed. Fruit formation is therefore decidedly *intermittent.*

This habit of producing fruit intermittently was observed by the writer in groups of plants grown in ornamental planting in the Botanical Garden during previous years. For the purpose of making special observations on the conditions involved in the intermittent production of fruit, a crop of 128 plants was grown in 1921. These plants were examined frequently throughout the entire period of bloom, and records were taken for each individual plant as to the character of the flowers opening at a particular time. At the end of the season, observations on the distribution of fruit in regard to the record for the flowers was made. Controlled self- and cross-pollinations were made on many plants.

Every one of the 128 plants produced many pods and the seeds were numerous, but without exception there was decided intermittency in the production of fruit. On several plants there was considerable irregularity in the distribution of pods, but for most plants the pods were in several groups quite as shown in figure 1.

The study of the flowers from day to day together with the results of controlled pollination showed that the intermittent production of fruit is due to repeated cyclic changes in the morphological character of the flowers, which in the course of the cycles give many grades of intersexes. The flowers of any individual plant varied from perfect or fully hermaphroditic flowers to flowers that were functional only as males or only as females, with also innumerable intergrades as to the relative abortion of pistils or stamens. As a rule, however, the loss of sex is decidedly *one-sided.* When the flowers are hermaphroditic or are female, fruit is produced provided pollination is accomplished; when the flowers are male only, no fruit is produced. The plants pass through alternating periods when the flowers are predominantly hermaphroditic or are female, during which they are productive of fruit, to periods when the flowers are predominantly or only male and fruitless.

The sex character of the flowers, therefore, varies in cycles, which variation makes the intermittent production of fruit a necessary result. The main raceme shown in figure 1 bloomed for a period of 107 days, and on it were produced about 250 flowers. During this time there were for this particular raceme five periods when hermaphroditic and female flowers were produced, with intervening periods when the flowers were staminate only.

In selecting material to illustrate these changes in sex, flowers opening on the same raceme at the same time were taken, the selection being made at a time when the variation was marked. It is, however, seldom that the variation on any one date represents the complete range observed for a plant during a complete cycle. As is shown in the figures, the sex organs

(pistils and stamens) when fully developed are large and conspicuous, and it is easy to observe variations in the degree of their development. The petals were removed from the flowers shown in Plate VI.

The three flowers shown in figure 2, Plate VI, were situated in a raceme in the succession shown and illustrate the range seen for the plant on the particular date when the photo was made; all the flowers were fully and very uniformly male; but the pistils were either normal and functional as in *a*, decidedly aborted and functionless as in *c*, or less conspicuously aborted as in *b*. On the particular date the flowers were varying in respect to femaleness. On other dates, however, maleness was quite as variable.

Maleness is well developed in all the flowers shown in figure 3, although the filaments vary in length and there is one stamen fully aborted in *b* and one in *c*. The pistils vary from the highly developed and functional as at *a* to the extremely aborted as in *d*. Figure 4 represents flowers of the same plant a few days later, showing extreme abortion in stamens of some flowers and some variation in the size of pistils, although all pistils were functional.

The pistils of the various flowers shown in figure 5 are either well developed (*a*, *b*, and *c*), or decidedly aborted (*d*, *e*, and *f*). The development of stamens is very irregular, and the extremes are seen for a single flower in the various grades as to length of filament and size, development, and dehiscence of anthers. Such irregularities as these are frequently seen, and for numerous plants the condition was more or less present throughout the period of bloom, with, however, no pod production for the flowers that had only aborted pistils.

Figure 6 shows two typical flowers of a plant on a date when the flowers could function only as females.

The four flowers shown in figure 7 show grades of abortion in both pistils and stamens and illustrate very well how the abortion tends to be one-sided, affecting first stamens and then pistils.

The many controlled pollinations that were made revealed that there was in these plants no limitation to fertility through physiological incompatibilities in fertilization. Every plant was highly productive of seed whenever pollen of dehiscing stamens was used on well-developed pistils either in self- or in cross-pollinations. Rudimentary pistils always failed to set seed. Pistils over 2 cm. in length usually produced seed.

Examination of pollen and tests for germination were made of pollen from all sorts of anthers. In large, well-developed anthers, 95% or more of the pollen grains appeared to be normal, and on a sugar-agar medium as many as 80% often germinated, producing tubes as long as 750 μ. In such rudimentary stamens as at *d*, figure 4, only a few shriveled, empty, partly developed pollen grains were present which did not even swell up when placed in water. In the large-sized but indehiscent anthers of short stamens as in *c*, figure 4, varying percentages of the pollen appeared to be normal, but in no case did the pollen of such indehiscent anthers germinate when

removed and placed on the same medium which gave good germination for the pollen of normally dehiscing stamens. There were many anthers that were partly dehiscent, that is, they opened to some extent, and the pollen thus shed was often viable in tests and productive of seed in controlled pollinations.

As a rule, the loss of sex for individual flowers was one-sided. When the pistil was rudimentary the stamens tended to be well developed as at *c*, figure 2. When the stamens were aborted the pistil was usually of good size as at *c*, figure 4. Occasionally, however, the pistil in flowers with aborted stamens was undersized as at *d*, figure 4, but cases of complete or extreme abortion of all stamens and of the pistil in the same flower were not observed.

The entire lot of plants were grown throughout under very uniform conditions which favored continued vegetative vigor, and only one generation has been critically studied. Development under conditions which affect differently the vegetative vigor and the length of the growing period may influence and possibly decidedly change the behavior in respect to cyclic changes, quite as such conditions are known to affect the sex of certain plants, particularly of *Arisaema triphyllum* (Pickett, 1915; Schaffner, 1922), from year to year. Definite evidence regarding the direct or indirect influences of environment and the somewhat synchronous changes of sex in the spider flower remains to be obtained.

At the close of the season, when the vigor of plants perceptibly wanes, all parts of the flower, corolla, pistils, and stamens alike, are uniformly undersized. Whether the last flowers that are produced on a plant that reaches old age before being killed by freezing temperatures are as a rule predominantly pistillate or staminate was not determined with certainty. On many such plants the last flowers were decidedly weak in maleness, but for other plants such flowers were decidedly male or bisexual.

Summary

In the cultures of *Cleome spinosa* grown for this observational study there was wide variation in the morphological character of the flowers in regard to the relative development of the two kinds of sex organs. The entire range of variations was seen among the flowers of a single plant, giving bisexual flowers, flowers that were functional only as males or as females, and many intergrading types. The variation from one extreme to the other was repeatedly cyclic, which condition results in the intermittent production of fruit.

All of the 128 plants grown under special observation were quite similar; all exhibited the extreme ranges of flower forms or intersexes; in all the production of fruit was more or less intermittent; none was exclusively staminate or pistillate.

Discussion

The alternative loss of maleness and femaleness in the flowers of *Cleome spinosa* and the recurring periodic changes in the sex of the flowers are to be regarded as phenomena of internal and biogenetic regulation closely related to those influences which determine the development of the plant as a whole.

It is, of course, well recognized that in plants as contrasted with animals there is continually the formation of really *new* organs from a persistent embryonic complex of cells and that this continues until the maturity and death of the plant as a whole. Internal and biogenetic relations of correlation and self-regulation, operating independently or in response to external influences, are hence repeated successively in determining the character of the new organs in the same fashion as they operate once for all in the animal. When there is in addition a long flowering period which overlaps and is coincident with the period of the most vigorous vegetative development, as is the condition in this robust annual *Cleome spinosa*, the conditions are most favorable for a study of the factors influencing the differentiation of sex.

The fact that the loss of sex organs in the flowers of the spider flower is very decidedly one-sided and qualitative is of special significance. When the stamens are aborted the pistil is as a rule functional, and in many cases it is well developed; when the pistil is aborted the stamens are often highly developed and functional. Here, as is the rule in plants, intersexualism does not lead to sterility of the plant or of a flower as a whole. Not a flower was found in which the pistil and all the stamens were extremely aborted, and rather rarely was the abortion of one sex accompanied by the decided abortion of the other sex in the same flower. Abortion of pistils was frequently accompanied by irregular abortion among the various stamens of a flower, but the same irregularity in maleness was also seen for flowers in which there was no abortion of the pistil (see the flowers of fig. 4). While the expression of sex in at least half of the flowers of a plant is decidedly one-sided and alternative, it is not mutually exclusive, for on every plant many bisexual flowers are produced.

It should be noted that the influences operate primarily and almost discriminatingly on the organs of sex. The pedicels, sepals, and petals are often uniformly well developed for all the types of flowers; but undersized flowers were to be seen (*c* of fig. 3, and *d* of fig. 4) in which the flower as a whole is undersized. Such cases, if more general, would suggest a direct relation to waning vigor and decreased food supply such as may be considered to be the direct cause of undersized flowers and of loss of sex in gynomonoecious forms at the end of a period of bloom. That the conditions are more intricate in Cleome is evident, for in a marked degree the extreme variations in sex are independent of any other visible change and the various grades of intersexes are present from the beginning of bloom.

Furthermore, the influences that affect the sex of a single flower often extend to groups of flowers. Thus there is a period of maleness, which is followed by a period of femaleness or of bisexualism, and this in turn is followed by maleness. Flowers in the same condition as to sex are grouped along the raceme. There is a series of cyclic changes all occurring during the period of continuous bloom.

These qualitative changes in sex in flowers of *Cleome spinosa* do not involve the transformation of organs of one sex into organs of the other sex after differentiation has begun, as is the case in many of the intersexes reported in animals (Goldschmidt and Poppelbaum, 1914; Goldschmidt, 1916; Banta, 1916; Lillie, 1917; Sexton and Huxley, 1921). Here the change is accomplished, as it is in dioecious plants, by the abortion of one or the other kind of sex organs. The relative position of each in the flower as a whole is maintained, but the differentiation giving male and female flowers (along with bisexual flowers) is as complete as is seen in many species of dioecious plants. The differential determination of sex in repeated cyclic alternative changes as they occur in *Cleome spinosa* shows to what degree the internal correlative differentiations in development may be extended to the organs of sex after the plant as a whole has passed from the exclusively vegetative to the reproductive stage. At the time of the transition to the reproductive stage, the change is not necessarily complete and discontinuous, nor are the flowers produced in succession necessarily of the same grades of sex. Even when the flowers appear to be morphologically the same there may be a decided cyclic change in their physiological character, as is the case with *Brassica chinensis* and *B. pekinensis* (Stout, 1922). The contrast between these species of Brassica and *Cleome spinosa* illustrates well the different types of sterility that may develop in plants and the different expressions of cyclic regulation of them. In these Brassicas there is frequently rather decided abortion of flowers at the time of transition from vegetative to reproductive organs; in Cleome no indication of such abortion is present, the first flowers to appear being often fully developed as hermaphrodites. In the Brassicas there is a somewhat extended period of flower formation with flowers all morphologically bisexual—but in which the physiological relations in fertilization may vary in a very definite and single cycle; in *Cleome spinosa* there is no variation in the physiological nature of stamens and pistils that are at all functional in so far as these may be tested by the relations of fertilization, but there is the cyclic alternation in the morphological development of the organs of sex. This comparison illustrates two rather widely different expressions of sex in its relation to fertility and sterility.

The conditions in *Cleome spinosa* favor the view that, as held by Yampolsky (1920), there is a general tendency away from hermaphroditism toward dioecism among the higher plants. In the persistence of perfect flowers in greater or less numbers along with those which are more or less

purely staminate or pistillate, *Cleome spinosa* is like most species which are in the transition stages toward dioecism. The alternate appearance of male, female, and hermaphroditic flowers in a raceme of course favors crossing, and when this alternation tends to be synchronous on all the branches of a plant, selfing is largely prevented except in the case of the hermaphroditic flowers.

In the spider flower, with its long flowering period and its alternation of maleness and femaleness in the racemes, it is evident that practically the whole vegetative feeding power of every plant is drawn upon for seed production. The conditions are markedly different, and we may consider them more highly adaptive to the demands of reproduction, than is the case in strictly dioecious plants in which seed production is confined to one of each pair of male and female plants. We may, perhaps, characterize the sex conditions in *Cleome spinosa* as effecting a sort of *super-dioecism* in that the conditions favor both reproduction and crossing for each individual.

Certain points regarding the determination of sex in the flowers of *Cleome spinosa* are clear. The conditions illustrate well the fact that the morphological differentiations of sex are fundamentally an extension of the phenomena of somatic differentiations. The expressions of differential qualities in leaves, stems, and flowers, with further differentiation of calyx, corolla, pistil, and stamens, with still further differentiations of tissue within each, are all recognized as one-sided, qualitative, and alternative expressions in protoplasmic units that are alike and which still remain alike in fundamental constitution. Even the physically qualitative division of germ plasm in the reduction divisions is found in regeneration experiments and in parthenogenesis not to be a direct and absolute condition in the alternation of generations. The theory of sex chromosomes decidedly fails in general application to plants, and even in animals, where its application seems most marked, sex is often intergrading and reversible, showing that there is alternative expression rather than alternative inheritance.

In *Cleome spinosa* it is evident that there are rather special and perhaps very specific stimulating and inhibiting influences which regulate the development of the sex organs. Whether these influences are substantive or more of the nature of stimuli, their action is cyclic and decidedly alternative. The results clearly show that sex of flowers is determined progressively as they are formed in response to regulation by internal biogenetic conditions.

New York Botanical Garden

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EXPLANATION OF PLATE VI

Each group of flowers is from the same raceme on the same date. Petals have been removed. The scale in inches shows the reduction from natural size.

FIG. 2. Three sister flowers uniform as to maleness but highly variable in femaleness.

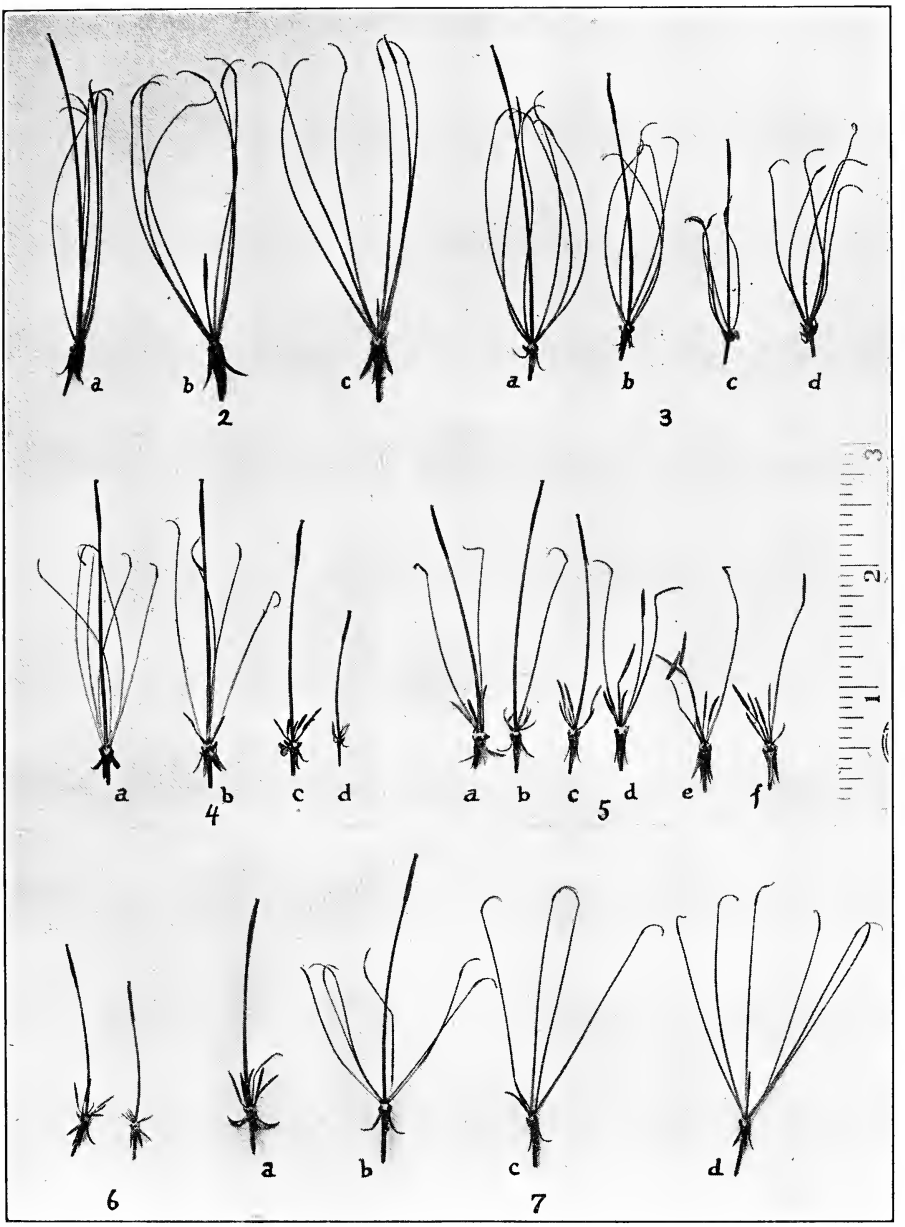
FIG. 3. The stamens of the four flowers vary as to length; one stamen in *b* and one in *c* are aborted; all others are highly functional. Pistils are functional in *a*, *b*, and *c*, but aborted in *d*.

FIG. 4. Four flowers from same plant as those of figure 3, several days later. Pistils vary as to length but all are functional. Stamens all excellent in *a*, two much aborted and indehiscent in *b*, all indehiscent in *c*, and all much aborted and containing no pollen in *d*.

FIG. 5. Pistils variable; in *a*, *b*, and *c*, fully functional; in *d*, *e*, and *f*, rather aborted. Stamens highly variable in each flower irrespective of the condition of the pistil.

FIG. 6. All stamens much aborted or rudimentary. Pistils somewhat undersized but functional.

FIG. 7. Abortion of stamens only, as at *a*, or of pistil, as at *c* and *d*, with a flower (*b*) fully hermaphroditic; all in bloom at the same time on the same raceme. Illustrates well the marked one-sided abortion of sex organs.



STOUT: ALTERNATION OF SEXES

INTERNAL DECLINE OF LEMONS

I. DISTRIBUTION AND CHARACTERISTICS [1]

E. T. Bartholomew, J. T. Barrett, and H. S. Fawcett

(Received for publication April 12, 1922)

Introduction

The term "internal decline" has been applied to a physiological abnormality causing the destruction of internal tissues in the lemon fruit, usually in the stylar end. The term as here used includes "blossom-end decay," "tip deterioration," "yellow tip," "dry tip," and other terms all applied locally by growers, packers, and shippers to the same trouble. When the study of this malady was first begun, the term was applied also to a browning of the "core" (placenta) and of the membranes (inner carpellary walls) covering the pulp segments of the lemon. Now it is applied only to the abnormality having the characteristics described in this paper.

Internal decline has been known by the lemon growers of California for at least 25 or 30 years, and the indications are that it is increasing in severity. Its seriousness fluctuates from year to year. For example, in 1920 in many of the groves as much as 10 to 60 percent of the fruit in an entire pick had to be culled out and discarded or sent to the by-products laboratory, while in the same groves in 1921 not more than 2 to 5 percent had to be discarded.

Experimental studies on internal decline were begun by the junior authors in 1915, and have been carried on intensively by the senior author since the summer of 1920. His discussion concerning one phase of his work on this problem will appear in an early number of this journal as the second paper in this series.

General Discussion

Distribution. Internal decline may appear in the fruit of almost any lemon grove in southern California, except that it seldom, if ever, occurs in groves located within a few miles of the coast. Groves situated within the hot inland valleys are especially likely to show a large amount of the trouble. A great variation in distribution may occur not only in different groves but in different parts of a given grove.

Varieties Affected. The two principal varieties of lemons grown in California are the Eureka and the Lisbon. Besides these there are a few other varieties which are grown in comparatively small numbers. Any of

[1] Paper no. 94, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

these varieties may be affected with internal decline. Data thus far obtained do not indicate any marked difference in varietal susceptibility. In some localities the Eureka is reported to be more susceptible while in other localities the Lisbon is said to suffer most, but so far the data do not confirm these reports.

Seasonal Appearance. The trouble usually appears with the beginning of warm weather in June and continues during the summer and fall until November or December. However, some lemons that are slow in attaining a desirable picking size may be allowed to remain on the trees for a longer period than usual, and consequently a few lemons showing internal decline may appear in the packing houses at almost any time during the winter or even in the early spring months.

Age of Trees. Internal decline has been found on trees of all ages ranging from 3 or 4 years up to 50 years. It is often the case that the fruit on young, thrifty trees with heavy foliage is worse affected than that on older trees with less dense foliage.

Age of Fruit. The terms "tree-ripe," "silver," "light-green," and "dark-green" are used in the lemon industry to designate lemons in different stages of maturity as indicated by their color. Tree-ripe lemons are those which remain on the tree until they have attained their mature yellow color; silver lemons are those which are picked at a time when most of the chlorophyll has disappeared from the fruit, leaving it a very light greenish yellow; light-green and dark-green lemons are picked according to standardized sizes. They are younger than the preceding kinds but are sufficiently mature for picking. When lemons are picked while they are yet green, they are either allowed to color naturally while in storage or are artificially bleached before being placed on the market. The lemons that are picked while green possess the most desirable commercial qualities because they have a higher acid content and are less susceptible to the attacks of diseases.

In some cases internal decline is found only in the tree-ripe fruit, but it often appears also in the silver, light-green, and dark-green fruit from the same grove. The tree-ripe fruit usually shows the greatest percentage of the trouble, sometimes as much as 95 percent being affected. However, in "bad years" as much as 60 percent or more of the green fruit in a given pick may be affected.

Symptoms

Green Fruit

External Symptoms. It is often impossible to determine without cutting the fruit whether or not it is affected. In some cases the trouble may be detected by a partial loss of luster at the stylar end. Another indication is the development of a yellow or orange-yellow color on a quarter or more of the stylar end while the remainder of the lemon is still green; even this appearance, however, is not a sure indication of internal decline.

Internal Symptoms. The first internal symptoms are usually found near the vascular bundles in the "nipple" of the peel at the stylar end of the lemon. Small cylindrical openings are found arranged in a circle within the ring of vascular bundles. It is evident that these openings have been produced by the collapse of the parenchymatous cells. A little later in the development the vessels themselves appear to be clogged with a pinkish to rust-brown deposit of gum. At this stage the vessels and the surrounding tissues often begin to break down, and finally the central portion of the peel of the stylar end of the lemon becomes a mass of gum having the characteristic pink to rust-brown color. In other cases the vessels are clogged with gum but there is very little indication of collapse in these or adjoining tissues. This is true for the examples shown in Plate VII. In conjunction with these conditions, pink to rust-brown splotches frequently appear at one or more places within the adjoining tissues (see *c*, Pl. VII). The tissues in these splotches, at this time, usually have a glossy appearance due to the formation of gum. At this stage the cells and the juice sacs of the pulp adjoining the peel at the stylar end of the lemon become affected, lose water, and collapse (see *b*, Pl. VII). There is very little or no discoloration of these tissues at this time. In comparatively rare cases the vessels in the center of the lemon are discolored and filled with gum all the way through to the stem end. This is usually as far as the trouble has progressed in lemons that are picked while light or dark green in color.

Silver Fruit

External Symptoms. Internal decline is not easily detected by external signs at this stage of development of the fruit. In many cases the more intensive coloring of the peel at the stylar end of the lemon is an indication that the abnormal breaking down of the internal tissues has begun. However, as is true with the green fruits, this indication often may be misleading.

Internal Symptoms. The loss of water and the collapse of the pulp cells and juice sacs at the stylar end continue. The progress is more rapid near the center of the lemon than out near the peel. It is especially rapid in the pithy core (placenta) that runs through the center of the lemon (see Pl. VII). As the fleshy pulp tissues dry out they may retain their normal color, but more often they assume a pinkish or light-brown color. In this stage of growth of the lemon it is found that, in those having internal decline, the breaking down of the tissue has progressed so far as to involve parts of the inner portion of the peel and perhaps a fifth of the adjoining pulp at the stylar end.

Yellow (Tree-ripe) Fruit

External Symptoms. Here again the signs are far from being infallible. The more intensive yellow or orange-yellow coloring of the stylar end, which may occur while the remainder of the fruit is still green, persists

after the fruit has become ripe and serves in many instances as an indication of internal abnormality. If the peel of the lemon is comparatively thin, the breaking down of some of the internal tissues will cause the formation of a depression at the base of the nipple on the stylar end of the lemon. In some cases the depression appears on one side only, thus causing the nipple to curve or bend over in that direction. These depressions are often, but not always, a sign that the lemon is affected and that it should be discarded.

In all the three classes of fruits the external signs are such that usually 50 to 90 percent of the lemons having internal decline may be detected and culled out.

Internal Symptoms. There is little or no indication of a further breaking down of the cells in the peel, but the pulp tissues continue to lose water and collapse as long as the fruits remain on the trees. When $\frac{1}{3}$ to $\frac{1}{2}$ of the stylar end of the lemon has become affected (see *d*, Pl. VII), an abscission layer usually forms in the stem and the fruit drops.

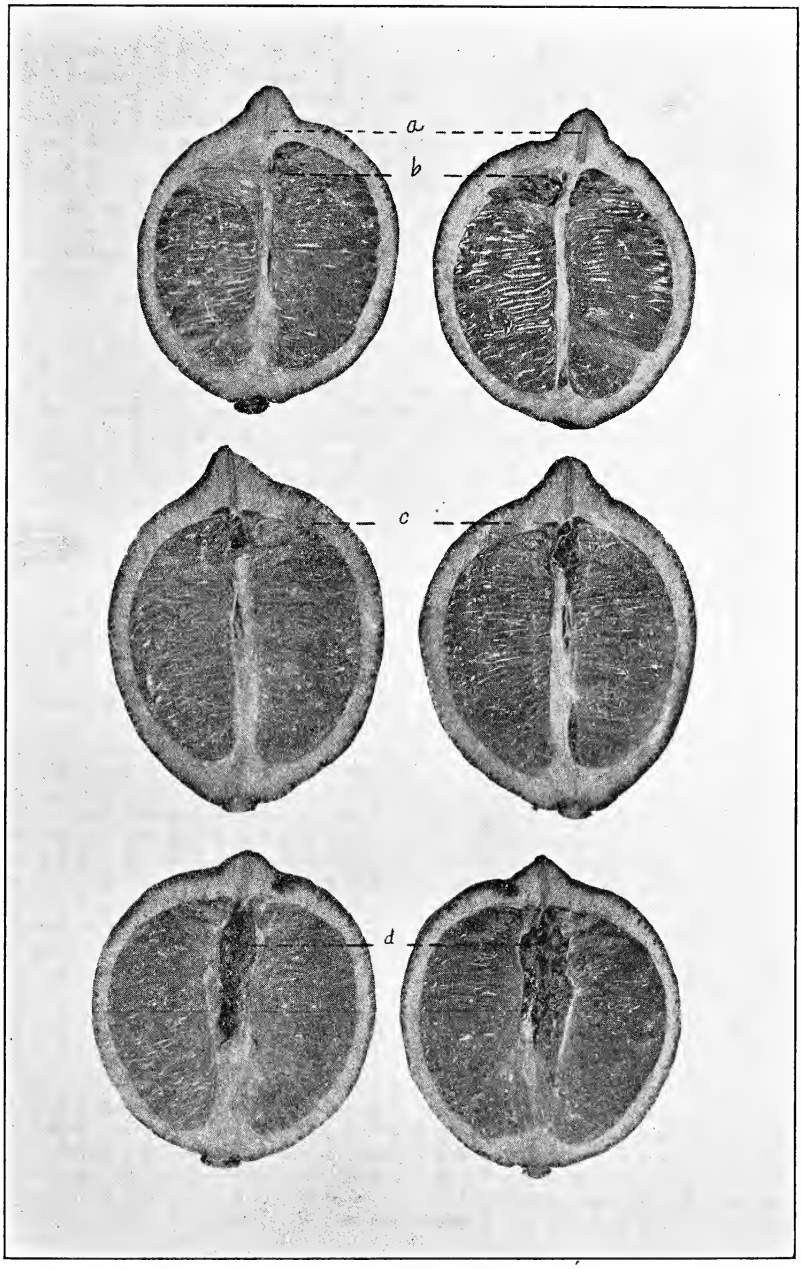
After the trouble has progressed to a considerable extent, the stylar end of the lemon becomes lighter in weight than the opposite end. On this account many of the badly affected lemons may be distinguished and culled out at the time of washing, because these lemons, unlike the sound ones, will float with the affected ends upward.

In the above description of the symptoms of internal decline, a typical example has been cited in which the collection of gum and the destruction of the tissues begin while the fruit is still green. As has already been stated, the malady may appear only in the silver or tree-ripe fruit. In such cases the course of development of the abnormal conditions is like that which begins while the fruit is still green, except that in some instances gum formation may be a little less abundant and discoloration of the tissues less pronounced.

EXPLANATION OF PLATE VII

a. Vascular bundles clogged with gum.
b. Initial collapse of pulp tissues.
c. Affected area in peel; vessels and adjoining cells becoming filled with gum.
d. Collapse of placental and adjoining tissues. The lemon usually falls from the tree at about this stage.

Note: The earlier stages of development of the malady cannot be satisfactorily shown in an ordinary photograph, and in fact none of the stages can be most advantageously shown without the use of a colored plate.



BARTHOLOMEW AND OTHERS: INTERNAL DECLINE

THE RELATION OF TEMPERATURE TO THE FUSARIUM WILT OF THE TOMATO[1]

EDWARD E. CLAYTON

(Received for publication April 19, 1922)

There are three different tomato diseases in the United States, supposed to be caused by three different species of Fusarium. They are the "summer blight" of California, the "yellow blight" of the Pacific Northwest, and "Fusarium wilt," which is one of the most serious tomato diseases in the southern states. Each of these geographical sections is subject to exceedingly high temperatures, and it has been observed that in periods of very high air and soil temperatures the Fusarium diseases cause the most serious damage. Edgerton (3) has recently published his observations on the relation of high temperatures to the Fusarium wilt disease. It is for this disease that the writer has attempted to determine definite temperature limits. The work herein described was carried on entirely under greenhouse conditions in the "Wisconsin soil-temperature tanks," the primary object being to study the effects of (1) different soil temperatures and (2) different air temperatures upon the development of the disease. The influence of other environmental conditions on the development of the disease, and detailed observations, hitherto unrecorded, as to the nature of the disease have been described also.

THE FUNGUS

The causal organism of the wilt disease under discussion is a vascular parasite of the tomato (*Lycopersicum esculentum* Mill.) which may live and retain its pathogenicity in the soil for considerable periods. Pure cultures of this fungus, designated as *Fusarium lycopersici* Sacc., were obtained from S. H. Essary of Tennessee, and in addition, isolations were made by the author from infected soil and infected plants sent from Maryland, Tennessee, Ohio, and Indiana.

When inoculations were made with these various isolations, it was found that, under greenhouse conditions at least, some produced much more serious wilting than others. After a series of preliminary inoculation experiments, therefore, one Indiana isolation was chosen for the temperature experiments. This strain was not so virulent a parasite as a Maryland isolation, but it produced the disease under a wide range of temperatures and was about equal in virulence to several other strains from Indiana,

[1] Investigations carried on at the University of Wisconsin. The work was done under advisory relations with Professors L. R. Jones and E. J. Kraus, to both of whom the author expresses his indebtedness for suggestions and criticisms.

Ohio, and Tennessee. In order to make sure that this Indiana strain chosen was representative among these isolations when inoculated plants were grown at different temperatures, a number of tomato plants were inoculated with it, an equal number with the Tennessee strain, and several pots of each were grown at several temperatures. The results in the two series were remarkably uniform. The amount of disease produced at each temperature was nearly equal in plants inoculated with the Indiana strain and in those inoculated with the Tennessee strain. Also, when pure cultures of the two strains were incubated together at different temperatures (for methods, see page 78), their respective optimum, maximum, and minimum temperatures for growth were found to be the same.

Identification. In order to insure confidence as to the identity of the organisms in hand, morphological and cultural studies were made following the methods of Sherbakoff (12). The Indiana strain was made the basis of these, with some comparative studies upon the other strains. The spore counts and measurements were made from 15-day-old cultures grown at 28° C. The following media were used: clover stems, tomato stems, potato plugs, rice, beans, potato agar (both 2 and 5 percent glucose), oat agar, malt agar, and water agar. It was found, however, that all the vegetative and reproductive parts of the fungus and all the color changes produced in any of these media were found on rice, tomato stems, and oat agar. The identification work consisted of observations as to the color and type of growth, the production and appearance of sclerotia and sporodochia, the character, septation, and size of macroconidia, and the method of bearing the chlamydospores.

These studies justified the conclusion that the organism in hand was *Fusarium lycopersici* Sacc., and that, although there are minor variations in pathogenicity and other characters, the Fusarium wilt disease of the eastern United States is probably all attributable to this one species.

Temperature Relations of the Fungus in Pure Culture

In order to determine the range of temperatures through which *F lycopersici* grows vigorously, pure cultures of the Indiana strain were prepared as follows: A small drop of a spore suspension made from a single-spore culture was placed in the center of each of a series of Petri dishes containing potato hard agar. These Petri-dish cultures were incubated at 28° C. for 24 hours and examined under the low power of the microscope. All cultures in which no spores had germinated, or which contained more than two or three germinating spores, were discarded, and three of the remaining dishes were incubated at each of 12 graduated temperatures, ranging from 4° to 38° C. It was found by daily observation that after six to eight days the fungous growth at temperatures approaching 28° C. entirely covered the surface of the dish. Plate VIII shows representative Petri-dish cultures after incubation for five days at each of the temperatures

chosen. As indicated by this photograph, the minimum for growth is 9–10° C., the optimum about 28° C., and the maximum 37° C., when the amount of growth is measured by the diameter of the colony. This trial was repeated with like results.

Similar cultures were made to determine the relation of temperature to size and septation of spores, the detailed results of which are not pertinent to this discussion. It was found, however, that, even when a single-spore strain of the fungus was used, there were marked differences in the average size of the spores as well as in the number of septa in the spores obtained from cultures grown at different temperatures.

Temperature Responses of the Normal Host

Two commercial varieties of tomato, Mangus and Chalk's Early Jewel, both susceptible to the wilt disease, were used in these studies. In each experiment uninoculated plants were grown as controls under the same conditions as the inoculated plants. These controls afforded an opportunity to study the growth of the host at different soil temperatures. It was found that the temperature range which, in sterilized soil under greenhouse conditions, proved most favorable for a vigorous development of these varieties was from 24° to 31° C. This range includes the temperature for the optimum development of the fungus (28° C.). The growth of the uninoculated plants is illustrated in Plate IX, *C*, a photograph taken one month after the plants had been transplanted at soil temperatures of 19°, 22°, 24°, 28°, 31°, 33°, and 35° C. respectively. As stated above, the plants grew most vigorously at temperatures of 24° to 31° C. and somewhat less vigorously at 33° C., while at 35° they lived but did not increase appreciably in size. In the cooler soil, growth decreased gradually from 24° to 19° C. and below.

Symptoms of the Disease

General Symptoms. The Fusarium wilt of the tomato as produced in the greenhouse at Madison was characterized by a progressive wilting of the leaves, often accompanied or preceded by a yellowing of the affected leaves. The wilting was sudden and permanent and developed most rapidly during bright, sunny weather, with little or no recovery during the night. The browning of the infected bundles could readily be seen in the cut stems. The roots appeared normal externally, though the bundles were browned as in the stems.

In well-developed plants three to four weeks old, the first external evidence of the disease was always the wilting of a lower leaf, this leaf being the first one supplied by the infected bundle or bundles. Frequently, one side of such a leaf wilted and the other side remained healthy for a time. In such instances, a cross section of the leaf petiole showed discoloration of the bundles on the wilted side only. The disease appeared next in other

leaves supplied by infected bundles, those leaves progressively higher up on the stem being attacked later. When, however, the fungus reached the succulent tissues of the upper portion of the plant, there was a sudden wilting of the entire plant. The stem and the older petioles were always the last to succumb and remained green and turgid for a time after the other parts had shriveled and blackened.

The symptoms varied somewhat, however, under conditions which were not favorable for the maximum development of the disease. Thus, if the plants were resistant, if the fungus was lacking in virulence, or if the environmental conditions were unfavorable to the growth of either host or parasite, the tendency was for the disease to appear as a slow blight rather than as a wilt. In this blight the leaves yellow and die slowly, while in the wilt they droop suddenly and die without yellowing.

Symptoms in Relation to Temperature. If a susceptible variety of tomato, such as Chalk's Jewel, is inoculated with a virulent strain of *F. lycopersici*, and the general growth conditions are optimum for the development of the disease, the following classification may be made on the basis of soil temperature:

First: Temperature optimum for the disease, *i.e.*, 25° to 31° C.—a sudden wilting which appears first in the lower leaves, then in those progressively higher up, and is rarely accompanied by yellowing of the leaves affected.

Second: Temperature just above or just below the optimum, *i.e.*, 33° to 34° C., or 20° to 24° C.—a wilting accompanied, and often preceded, by a yellowing of the leaves. The appearance may frequently be that of a slow blight rather than of a wilt for, as above stated, there is more yellowing than actual wilting, and the plants often show stunting of growth.

Third: At temperatures above 34° or below 20° C. there is no external evidence of the disease.

Fourth: In addition to the manifestations of disease mentioned above, the fungus may enter the host but penetrate the bundles in only the lower portions of the stem. This condition is often the result of a short exposure to temperatures favoring the disease, followed by a drop in temperature sufficient to check further development of disease. Plants thus infected are lighter in weight than uninfected plants grown under similar conditions, and the greater the amount of infection, the greater the loss in weight.

Growth Differences of Host and Parasite in Relation to the Disease

Since a parasitic disease is the result of interactions between host plant and parasite, external influences which modify the appearance of the disease are necessarily effective through the changes which they produce either in the host, in the parasite, or in both. If those complexes, called host resistance on the one hand and virulence of the parasite on the other,

were equally increased or decreased by a change in temperature, the degree of disease-expression might remain the same even though fundamental changes had taken place. It is more reasonable to expect that changes in temperature would not equally increase or decrease both the attacking power of the fungus and the resistance of the host, or, in other words, that pathogenicity might be increased out of proportion to resistance, or *vice versa*. It is also quite conceivable that a temperature change (*e.g.*, from medium to low) might increase the resistance of the host and at the same time reduce the pathogenicity of the parasite.

Since the amount of disease produced in the case of the Fusarium disease of tomatoes did vary at different temperatures, it seemed possible that a study of the temperature relations of the host plant and of the fungus separately might aid in interpreting the variations in the amount of disease produced under the different conditions.

In so far as the host plant is concerned, differences in temperature would affect the processes of food manufacture and also the utilization of the elaborated substances in respiration and other activities. Some microchemical work was undertaken in an attempt to discover the gross differences in composition which might be correlated with differences in the amount of disease. Reducing sugars, starch, and nitrates were measured quantitatively. It was not presumed that these materials were necessarily directly associated with qualities of resistance, but they are intimately concerned in the processes of growth, and the quantities present at any particular time vary widely with conditions of nutrition and environment. They might serve also as indicators of still other materials, possibly derived from them, which are more closely associated with resistance.

No definite interpretation can be made of the results of these chemical tests. When the plants were grown at approximately 17° C. there was no disease, while at 27° C. there was a maximum of disease, yet the quantitative differences in the compounds tested for in the two sets of plants were not marked. Again, with conditions of warm soil and cool air the lower portions of the plants held at the warm temperature were readily invaded by the fungus, which did not penetrate the upper parts surrounded by cool air. The chemical analyses, however, did not indicate marked differences in the composition of the tissues of these two regions.

There is a closer correlation, perhaps, between the temperature ranges of the host, the parasite, and the disease, considered separately, in the case of the Fusarium wilt of tomatoes than is ordinarily found. This correlation is especially marked at the optimum points, *e.g.*, it has been noted that the temperatures at which the host makes its most rapid growth range from 24° to 31° C. The fungus also grows most rapidly at these temperatures, having its optimum at approximately 28° C., and the disease is most destructive at soil and air temperatures of 25° to 31° C. There are, however, differences. Thus, the disease develops more rapidly at 31° than at 25°,

while the fungus grows with equal rapidity at 24° and 31°. Again, the growth of the fungus at 33° to 34°, the upper limit for the disease, was much less vigorous than the growth of the fungus at 19° and 21°, the lowest temperature at which the disease occurs.

Both host and parasite, taken individually, develop at wider ranges of temperature than does the disease occasioned by their interaction, the minimum and maximum temperatures being 9° and 37° C. for the parasite and approximately 10° and 36° C. for the host, while for the disease the minimum is 20° and the maximum 33° C. The relative rate of development of host, parasite, and disease corresponds in a general way as the temperatures are raised or lowered.

The Cause of Wilting and Death of the Host

The cause of wilting and the subsequent death of Fusarium-infected plants is still undetermined. It has often been assumed that the fungus actually fills the xylem vessels of the stem and thus limits the passage of water through the plant. Recently, both Haskell (6), studying the Fusarium wilt of potatoes, and Brandes (1), in his observations of the Fusarium wilt of bananas, have concluded that death is due to toxic substances produced by the fungus. There is some evidence that a toxic substance may be produced in Fusarium-infected tomato plants.

If it is true that the wilting results from plugging of the xylem tubes, then it should be possible to reproduce the symptoms by cutting away tissue, including xylem elements, in the corresponding parts of a healthy plant. As a matter of fact, although it is true that when one side of the leaf is diseased the infected vascular bundles are found only on the diseased side, yet, if the petiole of a healthy leaf is cut half-way through, the injured side does not wilt. There seems to be sufficient lateral diffusion of water to keep the entire leaf turgid. Similarly, leaves on one side of a stem can not be made to wilt by cutting through the vascular bundles on that side of the stem.

In Plate IX, *A*, is shown a plant which has thrown out vigorous suckers from an old diseased stem. This plant was transferred from a temperature of about 27° to one between 15° and 20° C. when the disease was in an advanced stage. The fungus was recovered from the very tip of the plant at the time of the transfer. All the leaves and the growing tip were killed, but in the cool temperature the old roots and stem were able to supply the moisture and other nutrient materials for a new top, which grew rapidly and appeared healthy. If the old stem had wilted because of plugging of the xylem tubes, it is difficult to believe that the change in temperature could have relieved this condition so rapidly. But while the temperature change would not affect the structural relations within the old stem, it would immediately influence such physiological reactions as the formation and activity of an injurious substance and the ability of the host tissue to neutralize it.

The type of wilt characteristic of the Fusarium disease also seems to differ from purely mechanical wilting in that there may be a complete and rapid killing of tissues and in that cells may regenerate and produce new shoots. In these respects it is more nearly comparable to the injuries produced in plants by the injection of poisonous compounds.

Free (4) has noted that the effects of poisonous compounds applied to the soil are much localized in the plant, and he suggests that this is due to the accumulation of these compounds in certain parts.

Of more direct interest are the experiments of Rumbold (10), in which toxic substances were injected into the trunks of chestnut trees. She found that

> The affected region extended up and down the trunk in a line whose width usually was but little more than the injection hole. The cells through which the solution passed acted like a blotter, with the result that the farther from the point of injection the more dilute was the solution and the smaller the injection stream.

And again, in a later publication (11),

> When a "killing" solution was injected the path was marked on the bark by vertical strips of dead tissues. Those twigs and branches whose vascular system entered this path were killed; often but one side of the branch was affected.

Gray (5) has found that many substances produce toxic effects when injected into tobacco stems. He found that in this herbaceous plant the localization of effects was quite as striking as in the case of the chestnut. Thus, a poisonous substance injected into one side of the stem passed up the stem rapidly and affected the leaves directly in its path; the lateral diffusion, however, was very slight. It was very common to find half a leaf killed and the other half remaining healthy, this effect being readily produced by injecting the poison just below and at one side of the petiole. In all cases there was marked discoloration of the bundles, and only parenchymatous tissue in immediate proximity to these browned bundles showed the toxic effect.

Thus the toxic effects of injection of poisonous substances and the killing of host tissues caused by the invasion of *F. lycopersici* appear very similar. It has been demonstrated by experimental work that with a warm soil temperature and a cool air temperature the infection of the plants takes place readily, the entire vascular system of the tap root and lower stem becoming infested with fungal hyphae and badly discolored. The cool air, however, prevented the fungus from extending up into the aerial parts, which remained apparently healthy both externally and internally, the plants making an excellent growth.

As to the nature of this toxic substance, there are several possibilities. It may be that it is a specific secretion of the fungus, or it may be a substance formed through the interaction of the host and the parasite. It has been amply demonstrated that even so-called normal root excretions removed in leaching may stunt plants grown in soil watered with these leachings.

With this disease, since the fungus is growing in the xylem vessels, toxic products could be promptly carried to the leaves.

To consider the manner in which the toxin affects the host, as a starting-point there is the fact that a wilt is produced. Caldwell (2), discussing wilting, says:

> Permanent wilting may result from the operation of either one or both of two factors: (*a*) decrease in the soil moisture content, (*b*) loss of water from the plant by transpiration. Attainment of the permanently wilted condition indicates a certain degree of reduction in the amount of water contained within the organism.

The primary effect of a toxic substance such as has been supposed to exist here would probably be upon the permeability of cell membranes. The permeability of the membranes might be increased, in this case facilitating loss of water from the cells, and, as a result, there might be a rapid increase in the rate of transpiration from the leaf as a whole. On the other hand, it is quite possible that permeability is decreased and that there is a slowing up of the movement of water from cell to cell. As a result of this, those cells farthest away from the veins might dry up completely while cells adjacent to the veins remained turgid.

Livingston (8) has shown that permanent wilting may occur with the stem and roots functioning normally, when the rate of transpiration from the leaves is greatly increased.

Methods of Experimentation

The experiments here described were all conducted in the greenhouses at the University of Wisconsin, and the Wisconsin temperature tanks were used in all cases in which soil temperature was controlled. In these tanks cylindrical cans of galvanized iron are immersed in water, the temperature of which is controlled by an electrical heating device. The most uniform infection occurred when a good loam soil was sterilized by steaming for three hours at six pounds' pressure and subsequently inoculated with *Fusarium lycopersici*. Neither the same loam not sterilized but artificially inoculated, nor imported naturally infected soils gave such consistent infection as was secured when soils were first sterilized and then inoculated. Unless otherwise indicated, freshly sterilized soil was inoculated by mixing with it a quantity of the mycelium of the fungus. For this purpose the fungus was so grown on rice in 500-cc. Erlenmeyer flasks as to obtain an abundant mycelial growth. The inoculated soil was then incubated at about 25° C. for a week or ten days in order to give the fungus time to permeate thoroughly and uniformly the soil mass. The galvanized-iron containers used in the Wisconsin temperature tanks were partially filled with sterilized soil, the upper four inches were filled with the inoculated soil prepared as described above, and the whole was thoroughly mixed. These containers were then incubated for several days at the temperatures to be maintained throughout the experiment. Thrifty tomato plants (4–5 inches

high) of the Mangus variety or Chalk's Early Jewel were transplanted into the containers, one plant of each variety being set in each container. Usually six such pots were held at each temperature until the infected plants at the optimum temperature for the disease were completely wilted. This time was a month or more, and meanwhile the progress of the disease was noted daily.

The plants used in these experiments were grown in three-inch pots until they were transferred to the inoculated soil. In transplanting, no considerable amount of soil was removed from the roots by washing or other methods, but the whole root system was kept as nearly intact as possible. A few of the smaller roots were necessarily injured, but infection was not more abundant in the plants bearing such roots than in those inoculated in the pots in which they were grown, and which, therefore, had no rootlets injured by transplanting.

Soil-temperature Experiments

1. *Experiments in which the air temperatures were uniform and the soil temperatures varied*

Three experiments, which for convenience may be referred to as I, II, and III, were conducted at a uniform air temperature and different soil temperatures. Only one of these experiments, however, need be described in detail, as the responses of the disease to different temperatures were the same in all trials.

Experiment III. Nine different tanks held at temperatures of 19°, 21°, 22°, 23°, 24°, 28°, 31°, 33°, and 35° C., respectively, were used in this experiment. Each tank accommodated six culture cans with two tomato plants in each can; three of these cans contained inoculated, and the other three uninoculated soil.

Table 1. *Results showing percentages of infection which occurred in plants grown in uniformly infected soil and held at soil temperatures ranging from 19° to 35° C. (Experiment III); when leaves were wilted and bundles blackened in stems, roots, and leaves, plants were counted as infected.*

Treatment			Results		
Temperature ° C.	No. Plants Used	No. Plants Infected	No. Days before 1st Infection	No. Days before Last Infection	Average No. Days before Infection
19	6	1	29	—	29.0
21	6	0	—	—	——
22	6	5	22	29	25.0
23	6	4	21	29	25.5
24	6	5	20	28	23.0
28	6	6	12	22	14.5
31	6	6	16	22	15.5
33	6	1	26	—	26.0
35	6	0	—	—	——

From this table it appears that the optimum soil temperature for the development of the disease is 28° to 31° C. (82° to 88° F.), the minimum 22° C. (71° F.), and the maximum 33° C. (91° F.). In this series one infection occurred at 19° C. but none was found at 21° C., a temperature at which one isolated infection had been secured in a previous experiment. The temperatures from 22° to 32° C., inclusive, resulted in the infection of about the same number of plants, the most favorable temperature for infection being distinctly marked, however, by the quickness and virulence of the attack. At 33° C. only one plant was infected, and at 35° C. none were infected.

The growth of the check plants is illustrated in Plate IX, *C*. The tops there shown are representative of the condition of the check plants one month after they had been transplanted to the temperature pots. At 35° C. these check plants maintained themselves and increased in size very slowly; 33° C. was a slightly more favorable temperature, and at 31° C. the plants grew luxuriantly. The growth at 28° and 24° C. was almost as great, and these plants appeared to be growing most rapidly at the time the experiment was stopped. The plants were growing thriftily at 22°, and equally well at 21°, while at 19° a slight decline was apparent.

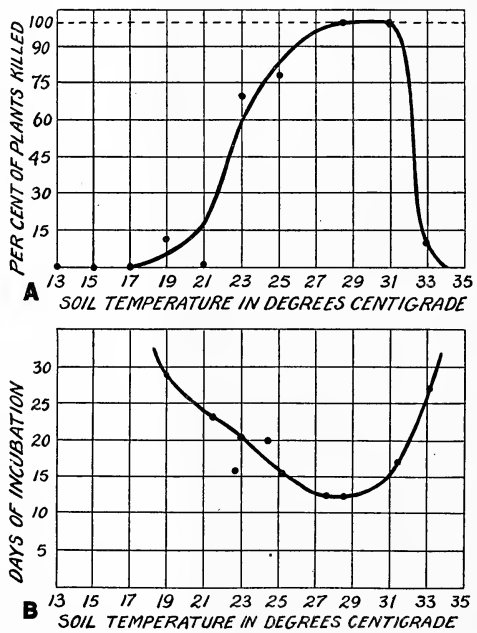


FIG. 1. The data for graphs A and B were secured in soil-temperature experiments II and III. Graph A shows the relation of soil temperature to the percentage of plants killed. The plants were exposed to the different temperature conditions for one month. Graph B shows the relation of soil temperature to the incubation period in days. Thus, it took much longer for the disease to appear with the soil at a temperature of 22° to 23° C. than with it at a temperature of 27° to 30° C.

These experiments showed, then, that there are ranges of soil temperature, approximately 14° to 20° C. and 34° to 35° C., which permit growth of both the plant and the fungus separately, but which do not favor the parasitic development of the fungus within the host.

2. Experiments in which both air and soil temperatures were varied

Early in these studies it became apparent that, although this fungus entered the plant through the root, it made its way into the aërial stem and did its damage in the above-ground parts. The roots appear to die last, the sequence being: leaves, then the main stem, and finally the roots. From previous work with soil temperature, the conclusion was drawn that soil temperature is a controlling factor only so long as the fungus is in the soil or roots, and probably exerts little or no influence after the fungus has grown up out of the soil. Thus it seemed logical to suppose that, once the plant parts above ground are diseased, air temperature alone might influence the progress of the wilt. In order to determine whether or not this is the case, experiments were conducted in which air temperatures as well as soil temperatures were varied.

Methods. Three sections of greenhouse were used in these experiments, with air temperature controlled at approximately 17°, 28°, and 33° C., respectively, by methods to be described in detail in a later publication. Unfortunately the ranges of air temperature procured were subject to considerable fluctuations, since in chambers where the bright sunlight is not counteracted in some way no ordinary means will prevent a rise in temperature. In reality, three ranges of temperature were obtained: a cool range of from 15° to 25° C., a warm range of from 25° to 30° C., and a hot range of from 30° to 35° C. In each of these sections a set of three constant-temperature tanks was placed. These tanks were maintained at 17°, 27°, and 35° C., to correspond with the air temperatures. Thus, by controlling both air and soil temperatures, nine combinations of soil and air temperatures were obtained (table 2).

Two experiments were conducted under these conditions of temperature. In Experiment IV the soil was steam-sterilized as usual and placed in the temperature-tank containers, and a spore suspension of the fungus was poured on the surface. Mineral wool, packed closely around the stems of the plants after they were set, served as insulating material. Two well-developed plants were used in each can.

In Experiment V, very small seedlings were transplanted into the sterilized soil of the containers and allowed to grow at 15° to 18° C. for a month, after which period the containers were transferred to the temperatures to be used throughout the experiment and incubated there for several days before the inoculum, a spore suspension, was added. A layer of mineral wool over the surface of the soil was used for insulation. At the beginning of the experiment the seedlings had attained a height of 3 inches and were

in a vigorous, thrifty condition. With two containers in each tank and three plants in each container, there should have been 6 plants at each temperature. However, the plants in one of the tanks died (table 2).

In each experiment plants were grown for one month in inoculated soil at the temperatures above stated.

Results of Experiment IV. Of the cool-soil-temperature plants, those grown in cool air and hot air (17° and 35° C.) showed no vascular discoloration; the isolation results also were negative. The plants grown in cool air (17° C.) and warm soil (27° C.) showed very badly discolored bundles at the base of the stem. This discoloration extended only a short distance up the stem, however, the entire stem which was surrounded by the cool air appearing perfectly normal externally. When the discolored bundles were plated out and incubated for a few days, an abundant growth of *Fusarium lycopersici* was secured in every case.

Plants grown in warm air (27° C.) and cool soil (17° C.) showed no evidence of vascular discoloration when sectioned. However, when the tissue from the base of the stem was plated out from each of the four plants, the fungus was recovered in pure culture from one of them. Warm air (27° C.) and warm soil (27° C.) gave 100 percent of disease, though only half the plants were completely dead at the conclusion of the experiment. Warm air (27° C.) and hot soil (35° C.) gave no evidence of vascular browning; the isolation results were also negative.

Hot air (33° C.) and cold soil (17° C.) produced neither disease nor vascular discoloration, but the fungus was obtained in pure culture from the base of one plant. Hot air (33° C.) and warm soil (27° C.) produced the disease in virulent form, one plant escaping infection while the other three were diseased or dead at the conclusion of the experiment. No results were secured with hot air (33° C.) and hot soil (35° C.), the plants dying soon after they were set in the pots.

As to the incubation period, the disease made its appearance several days earlier with the hot air (33° C.) and the warm soil (27° C.) than with the warm air (27° C.) and the warm soil (27° C.). The rapidity of the development of the disease, however, was about the same with these two combinations.

Results of Experiment V. The results of this experiment duplicated those of Experiment IV in the general temperature relations. However, the development of the disease was more marked than in Experiment IV because of the prevalence of bright, sunny weather.

At the end of the experiment all plants were closely examined for external evidence of disease. They were also sectioned to determine whether or not vascular infection had taken place. As table 2 indicates, under the heading "Discoloration of Vascular Bundles," infection of the xylem elements occurred in at least some of the plants at each temperature; but only when the infection progressed into the stem above ground were the usual

TABLE 2. *A résumé of the results of Experiment V, in which tomato plants were grown for one month in inoculated soil. In this experiment, as in Experiment IV, both air and soil temperatures were controlled.*

Temperature		Discoloration of Vascular Bundles	Wilting	Incubation Period of Disease	Comparison of Green Weight of Inoculated Plants with that of Check Plants
Air	Soil				
17° C.	17° C.	3 plants none 3 plants traces	None	—	—
17° C.	27° C.	6 plants marked	None	—	− 19%
17° C.	35° C.	3 plants none 3 plants traces	None	—	− 6%
27° C.	17° C.	3 plants none 3 plants traces	None	—	− 8%
27° C.	27° C.	2 plants marked	Marked	13 days	− 72% (4 plants out of 6 killed)
27° C.	35° C.	3 plants none 3 plants traces	Traces	20 days	− 28%
33° C.	17° C.	4 plants none 2 plants traces	None	—	+ 12%
33° C.	27° C.	3 plants marked	Marked	10 days	− 62% (3 plants out of 6 killed)
33° C.	33° C.	4 plants none 2 plants traces	Traces	25 days	− 28%

wilting symptoms observed. For example, when the soil temperatures were warm (27° C.) and the aërial parts were surrounded by cool air (17° C.), there was heavy discoloration at the base of the stem which neither progressed far above the soil line nor resulted in the usual wilting. The effect of the disease on the green weight, given in the last column of table 2, was calculated by using as 100 percent the green weight of tissue produced by a similar number of uninoculated plants grown under like conditions of temperature. As may be seen from the table, only two of the nine combinations of soil and air temperature so favored the development of the disease as to result in the death of plants. When soil temperatures were low (17° C.), infection was always slight; at the higher soil temperatures, when infection was abundant, it did not progress into the aërial parts of the plant if those parts were surrounded by cool air. On the other hand, when the soil temperatures were high enough (27° C.) to favor infection and the air was warm enough (27° or 33° C.) to favor the spread of the fungus through the aërial parts, the plants were badly diseased. Contrary to results obtained in previous experiments, the plants grown at soil temperatures of 35° C. and air temperatures of 27° C. showed some external

symptoms of disease. The symptoms, however, were not marked, consisting simply in a yellowing of the lower leaves, from which isolations of the fungus were made.

The Influence of Alternation of Temperature on the Development of the Disease

All through these experiments it has been observed that, while the wilt developed with a fair degree of rapidity when the temperatures were controlled, it was during bright, sunny days when the temperature of the house rose considerably that the disease was most virulent. Since this was noted with plants grown under conditions of constant soil moisture, the wilt cannot in this case be associated with water shortage due to drying out of the soil. Likewise, from the experiments with high air temperature it can be definitely stated that continuous exposure of the plants to air temperatures as high as those attained during the bright weather is not effective in increasing the amount of wilt. In the high-temperature (33° C.) plants, just as in the warm-temperature (27° C.) plants, the disease developed slowly during cloudy weather and with great rapidity during bright days.

Table 3 illustrates the degree to which the virulence of the disease is accentuated by clear, bright weather.

Table 3. *The Relation of Sunlight to the Amount of Wilting of Tomato Plants Produced by Fusarium lycopersici*

Date	Weather Conditions	No. Plants which Wilted during Day
4/21	Clear	0
4/22	Cloudy	5
4/23	Cloudy	4
4/24	Clear	7
4/25	Partly cloudy	5
4/26	Cloudy	1
4/27	Cloudy	0
4/28	Clear	6
4/29	Partly cloudy	14
4/30	Partly cloudy	8
5/1	Clear	9
5/2	Clear	16
5/3	Clear	17
5/4	Cloudy	0
5/5	Clear	22
5/6	Clear	43

From this record, kept during one of the soil-reaction experiments, it will be noted that on clear or partly clear days the disease was much more active than on cloudy days.

The retardation of the development of the disease during dark weather

and the rapid acceleration during bright weather have occurred consistently throughout the work. Thus, while during the dark month of January plants which were inoculated and held at a temperature of 27° to 30° C. developed the wilt and died, the incubation period was several days longer, and the progress of the disease was not so rapid as with plants held at the same temperature range in April.

From these observations it would seem that with a constant temperature conditions most favorable for the development of the wilt can not be attained. While soil and air temperatures of 27° to 30° C. seem optimum for the wilt disease, it is actually true that with a constant temperature of 27° to 30° C. and a sudden rise in temperature of 4° to 5° associated with bright sunlight, the wilt develops the most rapidly. It is, of course, entirely possible that the strong sunlight might have a direct effect quite apart from the temperature influence. However, for the present the problem will be considered only from the standpoint of temperature.

Experiment VI. In order to observe further the influence of alternation of temperature on the development of the disease, at the conclusion of the air-soil temperature experiments a lot of twenty plants which had been growing in inoculated soil at a low temperature for two months was brought into the medium temperature house and allowed to remain there for ten days. The plants all appeared healthy at the end of this time. They were then divided into two lots, of which Lot 1 was allowed to remain always at the medium temperature (about 27° C.) while Lot 2 was carried into the high-temperature (33° C.) house during bright, sunny days, in order that it might be exposed to excessive air temperatures for short periods toward the close of the normal incubation period. The normal incubation period under these conditions would be about 16 days. The results were as follows:

The incubation period of Lot 2, occasionally exposed to high air temperature, was two days shorter than the incubation period of Lot 1, which was maintained at a constant medium temperature (27° C.). The plants of Lot 2 wilted quickly, the progress of the disease being much more rapid than that in Lot 1. Thus, one week from the date of the first appearance of the disease several of the plants in Lot 2 were completely dead and most of the others were badly diseased, while in Lot 1 a number of the plants showed signs of disease, but in none was it well advanced.

Summary

This paper deals with (1) the relation of soil temperature to the occurrence of the wilt of temate caused by *Fusarium lycopersici*, and (2) the effect of combinations of different soil and air temperatures on the disease.

When the fungus was exposed in Petri-dish cultures to temperatures ranging from 4° to 38° C., the optimum for its growth was found to be about 28° C., although abundant growth was secured all the way from 18° to 31° C. (See Plate VIII.)

In three experiments, soil temperature alone was varied. The plants were grown in soil which was first sterilized and then inoculated with *F. lycopersici*. The temperature of the soil was controlled to secure a range from 14° to 35° C. The period of growth at these temperatures was four to six weeks. The optimum soil temperature for the disease was found to be about 28° C., though at 31° it developed almost as virulently. At soil temperatures of 33° or above and of 21° or below, the disease was practically inhibited.

Two experiments were conducted in which both air and soil temperatures were controlled. For this work the air in three greenhouse compartments was maintained at temperatures designated as cool (about 17° C.), warm (about 27° C.), and hot (about 33° C.), respectively. Three of the Wisconsin soil-temperature tanks were placed in each house and regulated to hold the soil temperatures, likewise, at 17°, 27°, and 35° C., thus permitting nine combinations of air and soil temperatures.

Air temperature was found to be as effective in controlling the appearance of the disease as soil temperature.

In only two of the nine combinations of air and soil temperatures did the disease make a rapid development, these two combinations being warm air (27° C.) and warm soil (27° C.), and hot air (33° C.) and warm soil (27° C.).

If the soil was kept too cool (17° C.) or too warm (35° C.), the disease did not develop, even with optimum air temperature.

If the air was kept too cool (17° C.) and the soil temperature was optimum (27° C.) for the disease, heavy infection occurred in the root and extended up into the basal portion of the stem. The plants continued to grow thriftily, however, and there were no external symptoms of the disease.

The temperature conditions of soil and air most favorable for the disease, as determined in tanks, are a soil temperature of about 27°, and an air temperature, after the fungus has established itself in the stem, of about 28° C., with short periods when the temperature suddenly rises to an excessively high point (33° or 34° C.).

Evidence accumulated during the course of these experiments leads to the belief that the wilting and death of plants attacked by the Fusarium wilt disease is due not to mechanical plugging of the xylem bundles, but rather to toxic action.

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EXPLANATION OF PLATES

Plate VIII

Petri-dish cultures of *Fusarium lycopersici* prepared by inoculating the center of each plate of potato agar with a single drop of a spore suspension of the organism. These were incubated for five days at a graduated series of temperatures ranging, as indicated, from 8° to 38° C. The highest temperature at which the fungus can develop, *i.e.*, the maximum temperature, lies between 35° and 38° C., the optimum for its growth is about 28° C., and the minimum between 8° and 10° C.

Plate IX

A. A plant inoculated and held at a warm temperature, favorable for the wilt (25°–30° C.), until the disease was well advanced, and then moved into a cool temperature (15°–20° C.) where the disease was checked. The stem on the right is the old main stem, all the leaves and the growing tip (*x*) having been killed by the wilt while the plant was still at the warm temperature; the growing tip, now dead and dry, may still be seen attached to this stem. The new growth, consisting of one shoot at the base of the stem and one higher up, was all formed after the plant had been exposed to the cool temperature. This new growth appeared perfectly healthy in every respect.

B. Representative plants taken from the temperature tanks in soil-temperature Experiment III. Note the prevalence of wilted plants between 22° and 31° C. and the healthy ones which grew at lower and higher temperatures. The optimum soil temperatures for the development of the disease, 28° and 31° C., stand out very conspicuously here.

C. Tops cut from control plants of the same experiment. They illustrate the vegetative development of uninoculated plants grown in sterilized uninoculated soil.

A comparison of photographs *B* and *C* shows the close correlation between the optimum temperature for the vegetative growth of the plant (22° to 31° C.) and the most pronounced development of the disease. Note also by comparison with Plate VIII that the same favorable temperatures hold for the development of the parasite.

Plate X

Representative plants grown in inoculated soil for 20 days in the temperature tanks of soil-temperature Experiment III. These plants were grown under exactly the same conditions with the exception of the soil temperatures, which were held at 23°, 28°, 31°, and 33° C. respectively. These plants show more clearly than those in Plate IX, *B*, the stunted and wilted condition of the stems and foliage at 28° and 31° C. This is in sharp contrast to the absence of any signs of the disease at either the lower temperature (23°) or the higher (33°).

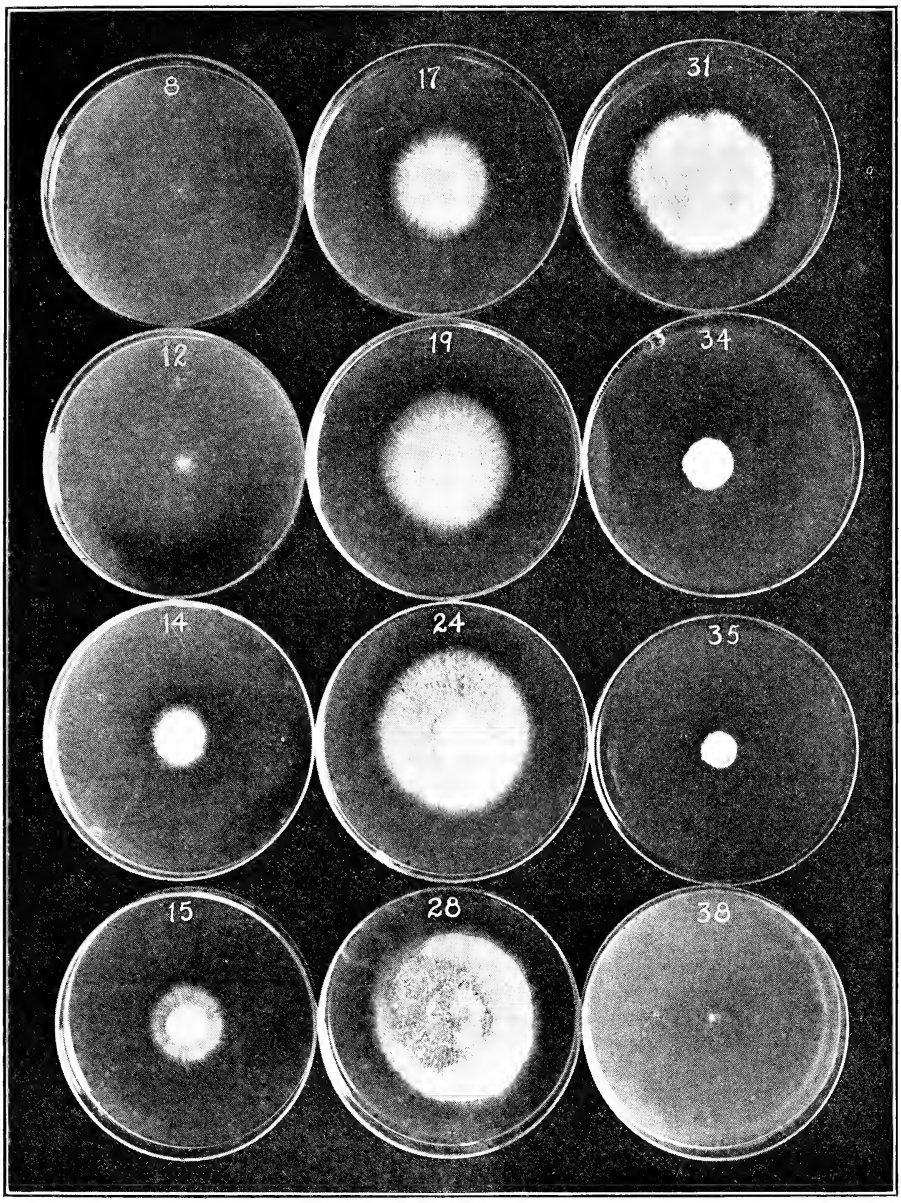
Plate XI

The Effect of Different Combinations of Soil and Air Temperatures upon the Tomato Wilt Disease

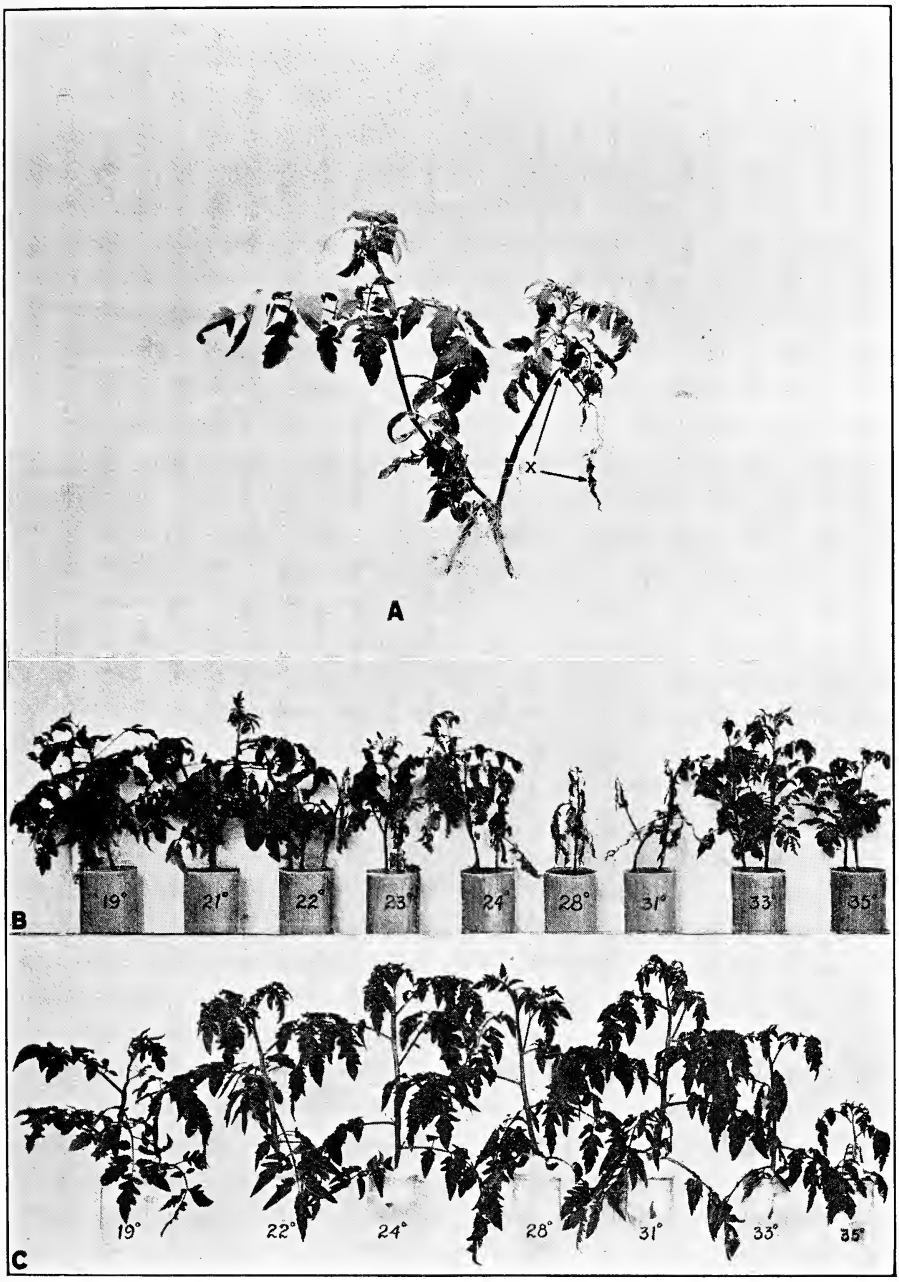
"A" is air temperature; "S," soil temperature

All plants alike were grown for one month in uniformly inoculated soil. Each pot is representative of a series, the different combinations of temperature conditions being shown in the labels.

Of the nine temperature combinations only two produced evident wilt symptoms, *viz.*, soil 27°, air 27°, and soil 27°, air 33° C. Especial attention is called to the middle plant in the upper row, grown in soil 27°, air 17° C. This soil temperature (27°) is the optimum for the disease; examination showed that the Fusarium had entered the roots, yet the disease was so inhibited by the low air temperature (17°) that the plants made a vigorous growth with no wilt. The plants at soil 17°, air 27° C., also deserve especial notice.



CLAYTON: TEMPERATURE AND FUSARIUM WILT



CLAYTON: TEMPERATURE AND FUSARIUM WILT

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CLAYTON: TEMPERATURE AND FUSARIUM WILT

AMYLASE IN THE SPORES OF *RHIZOPUS TRITICI* AND *RHIZOPUS NIGRICANS*

L. L. Harter and J. L. Weimer

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Introduction

The results obtained by a number of investigators have demonstrated that many fungi secrete enzyms, which diffuse out of the mycelium into the substrate, where certain cleavage products are formed.

However, Kopeloff and Kopeloff (3) seem to have been the first to study the enzyms of fungous spores. These workers found that the spores of *Aspergillus niger*, *A. sydowi*, and to a lesser extent *Penicillium expansum* and *A. flavus* contain an enzym which hydrolyzes cane sugar. Their results show that the deterioration of cane sugar may depend, in part at least, on the action of the spores of some of the mold fungi. The enzymic activity of the spores, previously killed by heating for 30 minutes at 63° C., was determined by suspending them in cane-sugar solution of known strength and determining the cleavage products by polarization. The rate of hydrolysis of the cane sugar was found to be correlated with the number of spores present in the system.

It is the purpose of this paper to record the data obtained relative to the occurrence of amylase in the spores of *Rhizopus nigricans* Ehrnb. and *R. tritici* Saito.

Methods of Experimentation

Since the mycelium of *Rhizopus tritici* has been shown to produce an amylase (2), it was imperative that the spores be completely separated from it before this enzym could be studied. Preliminary experiments showed that spores were produced abundantly and that they could be readily separated from the mycelium if the fungi were grown on sweet-potato decoction; hence this medium was employed. About 750 cc. of the solution were used in 2-liter Erlenmeyer flasks. On this medium a luxurious growth is made in from 5 to 10 days at temperatures ranging from 22° to 35° C. The spores, although abundantly produced in about 5 days, separated from the mycelium much more readily after about two weeks' growth; hence cultures of this age were used. At the end of the growth period, the fungus, which formed a thick felt floating for the most part on the surface of the medium, was carefully removed from the flasks. The bottom of the felt was held under a stream of running water in order to remove the culture solution. It was then floated top down in a dish of distilled water

and carefully agitated to remove the spores. By this method a considerable portion though not all of the spores were removed. To insure a complete separation of the spores from the mycelium which might have washed off into the water, this spore suspension was filtered through two thicknesses of a good quality of muslin, of about 20 threads to the centimeter. Most of the spores passed through the muslin while the mycelium was held in the cloth. At this time a microscopic examination of some of the filtrate was made to determine if any of the mycelium passed through the muslin. In case any of the mycelium was found mixed with the spores, the entire suspension was discarded. It is possible that some short mycelial threads escaped notice, but the writers feel certain that these were not sufficiently numerous to account for more than a very small part of the hydrolysis of the starch which actually took place. The spores were collected on a no. 2 Whatman chemically prepared filter paper, treated with a large volume of acetone for 10 minutes, and collected on a tarred filter paper (no. 2 Whatman). They were then immersed in ether for 3 minutes and dried to constant weight at a temperature of about 30° C. in a desiccator over calcium chloride. No germination of the spores could be obtained after treatment with the acetone and ether.

The hydrolysis of the starch paste was carried out in 150-cc. pyrex flasks for 18 hours. The temperature differed somewhat with the different experiments. The amount of spore material varied somewhat, but 100 cc. of starch paste were always used. About 3 cc. of toluol were used as an antiseptic. At the end of the digestion period the system was heated for 10 minutes in the autoclave to inactivate the enzym and was then filtered through a good grade of filter paper. The reducing sugars formed were determined volumetrically according to the method of Clark (1).

Only in a few preliminary experiments was an attempt made to separate the spores from the filter paper before they were used for the hydrolysis of starch. This process was attended by many difficulties and was finally abandoned. In all subsequent experiments the filter paper on which the spores were caught was included with the spores in the system. Before the starch paste was added the spores were suspended for 3 hours in 20 cc. of distilled water. Tests were made to determine if reducing sugars could be obtained from the filter paper, but only in one case, and that within the limits of experimental error, were any found. No reducing sugars were obtained from the starch when it was suspended in sterile distilled water.

Experimental Data

Experiment 1

The spores were obtained from a culture of *Rhizopus tritici* incubated at a temperature of from 22° to 25° C. The weight of the spores used in this experiment was not determined. Most of the spores were washed

from the filter paper in 30 cc. of water and extracted for three hours at 25°. After thorough shaking in order to obtain as uniform a distribution of the spores as possible, 10 cc. were pipetted off into each of two flasks, thus making three flasks each containing an equal amount of the spore suspension. To one flask (*A*) were added 40 cc. of a 0.5 percent starch-paste solution. A like amount of starch-paste solution was placed in the second flask (*B*), which was then steamed for 10 minutes to inactivate the enzym. To the third flask (*C*) 40 cc. of sterile distilled water were added. Hydrolysis was carried out for 18 hours at 38°. Results (average of several tests) expressed in milligrams of reducing sugars per 100 cc. of solution were obtained as follows: *A*, 105.3; *B*, 0; *C*, 0.

Experiment 2

Organism, *Rhizopus tritici*. The purpose of this experiment was to show that the enzym is produced in the spores at any temperature at which the fungus will grow. The exact quantity of spores used in these experiments was not determined. Cultures of the organism were grown at three temperatures, 9°, 30°, and 40° C. At 9° no fruiting took place, but at temperatures of 30° and 40° spores were abundantly produced. The spores were extracted for 3 hours in 10 cc. of water. Digestion of starch (50 cc. of a 0.5 percent starch-paste solution) was carried out for 18 hours at 38°, with the following results expressed in milligrams of reducing sugars per 100 cc. of solution: 30°, 163.8; 40°, 256.1. There were more reducing sugars formed by the spores from a culture grown at 40° than from one grown at 30°. However, this does not necessarily mean that spores produced at the higher temperature have a greater hydrolyzing power, since there may have been more spores present in the system. It does mean, nevertheless, that spores produced at such a temperature are capable of hydrolyzing starch to sugar.

Experiment 3

Experiment 3 represents the average of a number of parallel tests in which the amounts of the reducing sugars formed are expressed in milligrams per 100 cc. of solution. The exact weight of the spores was determined in each case, and the amount of starch hydrolyzed was calculated for 0.25 gram of spores.

TABLE 1. *Amount of reducing sugars formed in a solution of starch paste by the spores of Rhizopus tritici and R. nigricans, expressed in milligrams per 100 cc. of solution*

Temperature (° C.)	*Rhizopus tritici*	*Rhizopus nigricans*
22 to 25	335.35	103.23
30	—	215.00
38	189.49	—

The spores were prepared according to the method already described. They were extracted in 20 cc. of sterile distilled water for 3 hours. After the addition of 80 cc. of a 0.5 percent starch-paste solution, digestion was carried out for 18 hours at 38° C. The reducing sugars formed in the system

were determined in 10-cc. portions in duplicate. The results of these experiments are given in table 1. The spores were obtained from cultures grown at the temperatures indicated in the table.

It will be seen that the range of temperatures employed is somewhat limited. The object of these experiments was primarily to prove that a starch-hydrolyzing enzym was contained in the spores of *Rhizopus tritici* and *R. nigricans*. It was previously shown by Harter (2) that the mycelium produced amylase when grown at temperatures ranging from 9° to 40° C., but not uniformly so. The enzym was most active when the fungus was grown at 9° and least active when grown at 40°. Both of these fungi will grow and produce mycelium over a considerably wider range of temperatures than those at which spores are produced. As a matter of fact, *R. tritici* will grow at a much higher temperature than *R. nigricans*, while the latter will thrive better at a lower temperature than the former. The range of temperatures at which spores are produced is therefore limited in both organisms. *R. tritici* produced spores abundantly at temperatures ranging from approximately 20° to 38°, and *R. nigricans* from about 16° to 30°. Above and below these temperatures the quantity of spores was so small as to make the determination of the hydrolysis of the starch out of the question.

Within the limits of these experiments the results indicate that amylase is contained in the spores at any temperature at which they are produced. The same was also found true with respect to the mycelium. The data at hand are not sufficient to justify the conclusion that the amount of amylase contained in the spores is correlated in any way with the temperature at which the organism was grown.

Comparative tests of the hydrolyzing power of the spores and mycelium have shown that the enzym of the latter is more active than that of the former, when compared on the basis of unit weight. This holds true when the comparison is made between spores and mycelium grown at the same temperature.

Summary

1. An enzym capable of hydrolyzing Irish-potato starch paste to reducing sugars is produced in the spores of *Rhizopus tritici* and *R. nigricans*.

2. Within the limits of these experiments the enzym is produced at any temperature at which spores are produced.

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Washington, D. C.

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OBSERVATIONS ON THE CAUSES OF GREGARIOUS FLOWERING IN PLANTS[1]

William Seifriz

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One of the most interesting and fundamental of biological problems is that of the extent to which the life processes of an organism are influenced by the external environment. In the past, biologists have been quite content to rest secure in the belief that the most deep-seated characters in organisms are developed in the individual and transmitted from one generation to another little influenced, and certainly not determined, by the external environment of the organism. That so fundamental a character as paired eyes in vertebrates could in any great degree be influenced by a change in external environment was hardly conceivable until Stockard showed that if the eggs of the fish Fundulus are placed in sea water to which a little magnesium chloride has been added they develop into embryos with one medium cyclopean eye.

Equally interesting to the botanist have been the experiments of Garner and Allard (6), who were able by controlling the time of exposure of a plant to light greatly to lower or increase the age at which the plant reaches sexual maturity. Thus, the field aster, which commonly requires four months (May to September) to reach sexual maturity, was made, by decreasing the time of exposure to daylight, to bear flowers within a month after germination (by June 18). Still more remarkable is the fact that these same plants, instead of completing their life cycle by dying after flowering, as they would have done in the field, developed new axillary branches (on being restored to normal light exposure) and flowered a second time in September.

It is thus evident that certain characters of a deep-seated and fundamental nature which heretofore have been regarded as immutable, are relatively unstable and respond readily to changes in the external environment. It is, consequently, not surprising that some biologists hold that all "characters are of the nature of responses to environment" (7, p. 530), and that "every life process must to some degree be dependent upon the external world" (15, p. 285).

While it is difficult to deny the truth of these statements in the face of the remarkable experiments which have been performed, yet one wonders how far such a theory will carry us. We hesitate to admit that the external environment is in any way responsible for the fact that a pine seed develops

[1] Contribution from the Osborn Botanical Laboratory.

into a pine. We may even go further than this and maintain that the spreading form of the common poplar (*Populus nigra*) and the tall slender form of the Lombardy poplar (*P. nigra* var. *italica*) must, since the latter arose as a mutation of the former and since both forms breed true in the same environment, be due to the special nature of the protoplasm of the two trees and not to environmental factors. Those who hold the extreme view that "all characters are innate, acquired, germinal, somatic, and inheritable in exactly the same sense and degree" (7, p. 596) will find some difficulty in explaining the origin of fluctuations and mutations in certain individuals of a species when other individuals of the same species in the same environment remain true to type.

That external environment plays an important if not an inseparable part in inheritance and development is a fact of far-reaching significance. The problem of the biologist is to determine *to what extent* the heritable disposition of plants is susceptible to influence by the external environment.

In a study of this sort one must guard against concluding that an environmental factor is the *cause* of a vital phenomenon because it has been found to influence the phenomenon. Thus, from Garner and Allard's work we may conclude that length of exposure to light determines the *time* of flowering in certain plants, but we are not justified in regarding this environmental factor as the *cause* of flowering. The act of attaining sexual maturity is innate in all organisms, just as is senility. The *exact point* in the life cycle at which the individual shall become sexually mature is in some plants evidently sensible to external influence; *i.e.*, it is capable of being shoved this way or that by the environment.

The problem of determining the environmental factors which influence the flowering of plants assumes a special interest when considered in reference to those plants which flower only after a period of many years of purely vegetative growth. Particularly interesting does the problem become when applied to those plants in which all the individuals of a species throughout a given region attain sexual maturity simultaneously. The bamboos (many of them) belong to this category.

Drought as a Cause of Gregarious Flowering in Bamboos and Palms

One of the oldest theories of the cause of gregarious flowering in bamboos is that this simultaneous anthesis is occasioned by drought (18, p. 251). This theory is still held by many biologists. Some (8, 16) have advanced another hypothesis, namely, that periodic flowering in bamboos is the result of a depletion of nourishment. Both theories are opposed to that which would attribute this sex phenomenon to a heritable factor in the plant.

The chief criticism to be directed against such theories is that their supporters have failed to look further than the particular group of plants and the special set of environmental factors under observation.

That bamboos have flowered gregariously in India immediately after a drought is not to be doubted. Droughts are of such frequent occurrence in India that it would be surprising if they did not occasionally coincide with the flowering of bamboo forests. It is also possible that severe dry weather may have some *slight* influence on the *exact time* of flowering. When many individuals of a species flower simultaneously immediately after a drought, scientist as well as layman is likely to associate the two phenomena. The occurrence of each phenomenon separately passes unnoticed. But even if our data should warrant the conclusion that the gregarious flowering of a particular species is occasioned by drought in a certain locality, what are we to do with the fact that the same species flowers in another locality where there is no drought? *Bambusa arundinacea*, for example, flowers not only in India, where it is subjected annually to a severe dry season and occasionally to a drought, but also in Buitenzorg, Java, where dry seasons are practically unknown. Then, too, we have the interesting fact that other species of bamboo flower gregariously following an unusually *wet* period, as did, for example, the climbing bamboo, *Chusquea abietifolia*, in Jamaica in 1918 (20). Still other bamboos show no periodicity at all and flower sporadically without any apparent relation to climate. This is true of the Philippine bamboos in general, among which no case of simultaneous flowering of many individuals is known, although these bamboos have been under scientific observation for nineteen years.[2]

So far as I have knowledge, no one has actually investigated the rainfall data of the country where bamboos flower gregariously in order to ascertain, first, whether or not the dry season of the particular year in question was one of sufficient severity to warrant its being regarded as the direct cause of the gregarious flowering of the bamboos; and, second, whether or not, in case a drought did precede the particular flowering period under investigation, other flowering periods of the same species of bamboos in that country (and in other countries) were also preceded by droughts.

The meteorological conditions prevailing in India are so extreme that one must be thoroughly aware of them in order to investigate intelligently a question such as that under consideration. The greater part of India is almost rainless for about seven months of the year. It is not an uncommon occurrence for no rain whatever to fall at certain stations during eight months of the year. The absence of rain during such a normal dry season cannot, of course, in any sense be regarded as a drought. What rain does fall during the dry season (averaging 0.2 to 0.3 of an inch a month) is of little consequence to plant life. Vegetation depends solely on the monsoons, which occur from June to September in Hyderabad and the Central Provinces, for example, and from October to December in Southeast

[2] Dr. E. D. Merrill, Director of the Philippine Bureau of Science, has kindly given me this and other valued information.

Madras. If the monsoon rains fail, severe consequences follow. This is what happened in 1899 and 1900. There was a phenomenal failure of the rains over a large part of India during the 1899 monsoon (June to September). As a consequence, a disastrous drought prevailed in the early months of 1900. *Both* immediately *before* and some time after this severe drought large bamboo forests flowered in northern India.

In the Indian Forester for 1899 there appears the following note (10, p. 178):

> The flowering of *Bambusa arundinacea* is reported . . . to be general this year in the Angul Division[3] of the Bengal Presidence.

The drought of 1899–1900 above referred to could have had no effect on the life of these bamboo forests, since the bamboos were in flower (in April) before the failure of the monsoon rains (June to September), the disastrous consequences of which could not have been felt by plants until early 1900. In 1898, the year preceding the flowering of the bamboos in the Angul District, the monsoon was normal—at Angul (41.29 inches) slightly below the average (49.34 inches), and at Bissipara (58.49 inches) somewhat above the average (55.07 inches). For the four preceding years (1894–1897) the total annual rainfall in the Angul District was either just at the average or considerably above average—never below. We cannot, therefore, accredit the general flowering of *Bambusa arundinacea* in the Angul District of India in 1899 to a drought.

In the Indian Forester for 1901 (11, p. 126) is reported

> The flowering on a large scale of the ordinary bamboo (*Dendrocalamus strictus*). The area over which the flowering extends is estimated at 1200 square miles, and in this area, although a few clumps here and there have escaped, the phenomenon is universal.

The flowering occurred in the Chanda District of the Central Provinces of India. The rainfall data for 1900 from sixty-seven observation points in the Central Provinces indicate that the monsoon of that year was all that could be desired. For example, the total annual rainfall for forty-five of the sixty-seven stations was, in 1900, above the annual normal (in several instances nearly double the average annual precipitation).

It would hardly seem necessary to go further back than the favorable monsoon of 1900 in our investigation of meteorological conditions and of their bearing on the flowering of the bamboo forests in the Central Provinces of India in 1901. This statement is based on the assumption that the visible effect of a climatic influence which is potent enough to affect the physiological state of plants is likely to become evident within a year after the climatic factor came into existence. To what extent such an assumption is justifiable is an open question. Brandis (1, p. 14) believes "that such stimulating conditions must act upon the plant at least a year before the flowering actually takes place." Yet in the same article he refers to

[3] The official meteorological designation of the "Division" in which the Angul "District" occurs is the "Chota Nagpur Division" of the Bengal Presidency.

the observations of Kurz in Burma where an unexpectedly large number of bamboos were collected in flower "*during* [italics mine, W. S.] the two dry seasons of 1868 and 1869." Brandis further quotes Kurz as stating "that in the Calcutta Botanic Garden there never had been so many species in flower as in 1874, which was a year of great drought" (10, p. 14). It will be noticed that the abundant flowering of the bamboos in the Calcutta Garden occurred *in* 1874, *i.e.*, during the year of great drought, and that the supposed stimulating conditions did not act upon the plant "at least a year before the flowering" actually took place, as Brandis elsewhere maintains must be true.

To go further back than the favorable monsoon of 1900, *i.e.*, to go back more than a year previous to the flowering of the bamboos in 1901, involves the question whether or not meteorological conditions, occurring more than a year before the appearance of the vital process which they are supposed to initiate, can be taken into consideration. It seems hardly likely that bamboo plants which flowered in early 1901 and whose flowering was preceded by a favorable rainy season in the mid-year of 1900 should have flowered as a result of a drought in early 1900. Not only Kurz, whom Brandis quotes, but others who have ascribed the gregarious flowering of bamboos to drought, have spoken of the flowering as occurring in *times* of drought.

Owing to the severity of the drought of 1899–1900, we cannot altogether ignore the possibility of the unfavorable climatic condition having initiated in the bamboos of northern India a physiological process which did not become externally evident until a year and a half later (in the simultaneous flowering of the bamboo forests in 1901). Once the marked change in the physiological state of the plants was initiated, a subsequent favorable climatic condition (the monsoon of 1900) would be of no effect.

The question cannot be conclusively answered. It is possible that the extreme drought of 1899–1900 of the Central Provinces of India had a telling effect on the bamboos of that region. But that the drought was the *cause* of the simultaneous flowering of the bamboos is not, in the face of other data, a possible deduction. The most that can be said is that when bamboos are near their time of reproduction an unusually dry season may have the effect of accelerating the formation of flower buds.

Whatever our decision regarding the possible effect of the drought of 1899–1900 on the general flowering of *Dendrocalamus strictus* in the Central Provinces of India in 1901, we have the definite fact that *Bambusa arundinacea* flowered gregariously in India in 1899 in the absence of a drought for at least five years previous to the flowering.

Another interesting bit of evidence against the theory that drought is the cause of gregarious flowering in bamboos of long life cycle is to be found in the behavior of an immense bamboo forest region in Burma. The bamboo in this case is of another species (*Bambusa polymorpha*) than the two (*Dendrocalamus arundinacea* and *D. strictus*) we have just been considering.

The sexual cycles of *D. arundinacea* and *D. stric us* are about thirty-two years in length. *Bambusa polymorpha* is known to have a very long life period. I know of no authentic record of two successive flowering dates.

In the Indian Forester for 1903 appears the statement that "the last recorded flowering of the Kyathaung was . . . in 1853" (13, p. 244). The flowering of this bamboo, *Bambusa polymorpha*, was expected to recur shortly after 1883 on the general belief that the life of bamboos is about thirty years. The flowering of *B. polymorpha* in the forests of Burma has not yet occurred. Certain "signs" of an expected flowering have from time to time been seen. These signs refer to the well-known habit which bamboos have of producing no new shoots in the year of flowering.

The bamboo forests of *B. polymorpha* in Burma may be reckoned by hundreds if not by thousands of square miles. In this extensive region of bamboos there have been, since 1883, one or two false alarms of gregarious flowering when a clump or two has blossomed. In 1918 and 1919 an area of several hundred acres in two or three distinct but neighboring blocks in the Tharrawaddy Division flowered gregariously.[4] (The plants of *B. polymorpha* were at this time sixty-five years of age.) This was thought to be the forerunner of a general flowering, since the flowering of odd clumps is considered to be an indication that the flowering of the whole area is imminent. But so far no general flowering has taken place.

During their sixty-eight years of existence the bamboos of these forests have endured many droughts which apparently have had no effect whatsoever on the sexual maturity of the plants. For at least the latter half of their life the bamboos must certainly have been mature enough to respond to an external stimulus, if this stimulus is of such a nature as to exercise any prominent influence on the sexual life of the plants.

We have so far seen, first, that bamboos of long and rhythmic life cycles reach sexual maturity when experiencing only the normal annual dry season of the tropics; and second, that other bamboos of long periodicity have for sixty-eight years failed to attain sexual maturity even though they have experienced many seasons of both normal dry weather and drought. There now remain to be considered those instances in which flowering takes place in the entire absence not only of drought but even of a typical tropical annual dry season.

The most striking instance of this is the behavior of the bamboos at Buitenzorg, Java, where droughts are unknown and dry seasons are few and far between. Before passing on to the Buitenzorg data it will be interesting to consider in more detail the similar case of the climbing bamboo, *Chusquea abietifolia*, already referred to. This bamboo, a slender scandent form, occurs in great abundance throughout the mountains of Jamaica. The high altitude regions of Jamaica receive abundant moisture

[4] I am indebted to H. R. Blanford, Esq., O. B. E., Government silviculturist at Maymyo, Burma, for this information.

at all times of the year. Some few arid localities occur, but they are small and infrequent above 4000 feet. Relatively dry weather may come occasionally at high altitudes, but never a drought above 5000 feet, the elevation at which Chusquea grows. *Chusquea abietifolia* flowered gregariously in the mountains of Jamaica in 1918 (20). Over an area ten miles in length (investigated by the writer) the trails were, in 1919, lined with dead tangled masses of this climbing bamboo. The two years immediately preceding the flowering of Chusquea were (at Cinchona) unusually moist ones. It is interesting to note that specimens of *Chusquea abietifolia* sent to Kew, England, in 1884, a year prior to the last previous flowering of the plants (in 1885, the life cycle of Chusquea being thirty-three years), flowered simultaneously with the plants in Jamaica.

The behavior of Chusquea in Jamaica stands in further opposition to the belief that lack of moisture may cause flowering in bamboos in that it does not support the statement of Brandis that "there are indications, that in dry stony places . . . bamboos flower earlier and more abundantly" (1, p. 662). It was in just such places that the only green living specimens of *Chusquea abietifolia* were found in the mountains of Jamaica. On an exposed hot and dry spur, sparsely covered by a typically xerophytic vegetation, were growing a quantity of old, green, and thriving specimens of the climbing bamboo. Immediately below this dry spur on which living old plants of Chusquea were growing, there is a moist, cool ravine. Here no adult living specimens were found, but there existed instead the condition prevailing generally throughout the mountains: old plants were dead and growing seedlings were abundant. The old living specimens on the arid spur above were not in fruit. Flowering had not taken place earlier, as Brandis suggests, but, on the contrary, had been delayed. Possibly the climbing bamboo had in this more arid region assumed an altered life cycle.

In comparing the behavior of the bamboos at Buitenzorg, Java, where droughts are unknown and dry seasons are infrequent, with the behavior of the bamboos in India and Ceylon, where dry seasons of several months come annually and droughts occur frequently, it will be well to consider with the bamboos the equally instructive case of the talipot palm, *Corypha umbraculifera*, which, like some bamboos, has a long vegetative period at the expiration of which the palm flowers and dies. Ordinarily *Corypha umbraculifera* does not flower gregariously nor at a fixed age, as do certain bamboos. Consequently, when many specimens of the talipot palm do flower simultaneously, one is likely to suspect the presence of some external factor which has aroused the palms to sexual activity.

The most remarkable case of simultaneous flowering of plants of which I know is that which recently occurred at Peradeniya, Ceylon. In the annual report for 1918 of the Director of the Royal Botanic Gardens at Peradeniya (4) there appear the following three notes:

Seven out of the sixteen talipot palms (*Corypha umbraculifera*) forming the avenue,

started by seed sown *in situ* in 1881, commenced to flower in June, and continued in blossom until the end of the year, being at their best in October–November [Pl. XII, fig. 1].

Bauhinia anguina, a very large woody climber with peculiar alternately compressed chain-like stems, has this year fruited for the first time on record at Peradeniya. Trimen, in his Flora, states: "Very rare, flowers and fruit not seen."

The flowering of the giant bamboo (*Dendrocalamus giganteus*) is not now the rare event in Ceylon it used to be. Nine clumps produced flowering stems during the early dry months of the year. . . . None of these clumps have died. Eleven clumps of the "male bamboo" (*Dendrocalamus strictus*) also flowered profusely early in the year. Of these, five clumps have died in consequence.

To this is to be added the interesting fact that at the same time that the talipot palms were blossoming in the Gardens there were counted from one observation point elsewhere in Ceylon two hundred talipots in flower. So extraordinary a concurrence of the profuse flowering of four species of plants, all of whose life cycles are very long—in the case of the two bamboos about thirty-two years, of the talipot palm nearly forty years, and of the liane Bauhinia so long that there is no record of it—is indeed an event that forces one to search for some possible environmental factor which might be responsible.

The annual dry season of 1918 was in Ceylon longer than usual, sufficiently long to be locally termed a drought. The remarkable flowering of so many talipot palms throughout Ceylon and the simultaneous flowering of three other species of plants of long sexual periodicity was attributed to this drought.

Three objections can be raised against such a deduction. First, the drought of 1918 was a relatively mild one. The total precipitation for the four months (January to April) of the dry season was, to be sure, below the average for this time of year (12.9 inches in 1918 as compared with a normal of 17.05 inches for these four months, all averages being based on twenty years' records from 1901 to 1920); yet the difference is not very great. Furthermore, if we review the records of the years immediately preceding, we see that the dry season of the second year before the flowering of the talipots and bamboos in the Gardens was also below the average; not quite so low for the four months of the dry season as in 1918, but much lower for January, when but 1.0 inch of rain fell (in 1916) as compared with 5.23 inches in 1918. And in February, 1916, there was but 0.03 inch of precipitation. One would expect these two very dry months of 1916 to have a more telling effect on plant life than the dry season of 1918. If we go further back we find that there was a drought in 1903, and again in 1905, of much greater severity than that of 1918, especially the latter one (1905) when the total rainfall for the four months January to April was the lowest on record for twenty years (1901 to 1920), namely, 4.9 inches or nearly one third that of 1918. In 1911 there occurred at Peradeniya a drought which, because of its duration, was more severe than any so far mentioned. In this year there fell during the *five* months of January to May but 12.78

inches of rain as compared with a normal average of 21.95 inches for this period. Especially trying must this drought have been on plant growth in view of the fact that the precipitation in April, which usually ends the normal dry season, was less than half the average, while in May there fell but 0.75 inch of rain as compared with a normal precipitation for this month of nearly five inches (4.90). In 1911 the talipot palms were but seven years younger than in 1918, *i.e.*, they were thirty years old, not too young to respond sexually to an external stimulus of some force.

Through all these droughts the six talipots, with others in the Peradeniya Gardens and hundreds throughout Ceylon, the twenty clumps of bamboo, and the liane Bauhinia, grew on without flowering. It is therefore hardly likely that the relatively mild drought of 1918 had any influence on the flowering of these plants.

The second objection to be raised against the conclusion that drought caused the flowering of the plants in the Peradeniya Gardens is that nine out of the sixteen talipot palms did *not* flower, and these nine were of the same age and had been growing under the same conditions as the seven which did flower. Obviously, if flowering was the direct result of drought or of any other climatic factor, the seven palms which were affected must have been in such a physiological state as to be susceptible to the influence while the other nine palms were not in such a state. That is, if drought is a factor it is a secondary one, the physiological condition of the plant being the primary determining factor.

If we conclude that the ultimate cause of the time of attaining sexual maturity lies in the hereditary disposition of the plant, the interesting question arises, Why did seven of the talipots flower and nine not, since all in the avenue were of the same age? We can only attribute this difference in behavior to individual differences in the germ plasm, concerning the causes of which we know nothing. The age at which *Corypha umbraculifera* reaches sexual maturity is not the same in all individuals.

The final and most convincing evidence against the hypothesis that drought is the *direct* cause of flowering, or even a factor of any great significance in the flowering of certain palms and bamboos, is the behavior of another talipot at Peradeniya and of a talipot and the bamboos at Buitenzorg. The talipot in question at Peradeniya is one which flowered some years ago, in 1903 (fig. 2). For four years (1899–1902) previous to the flowering of this palm at Peradeniya the average annual rainfall was, in each of these four years, above the normal average. In 1902, the year immediately preceding the flowering, the total annual rainfall was approximately one third above the normal average. It is quite evident that the flowering of this talipot can in no way be attributed to drought.

When the many talipots in Ceylon were blossoming in 1918, the only old specimen of this palm in the gardens at Buitenzorg was also in flower (fig. 3). At Buitenzorg there is no such thing as drought. The writer was

there during the so-called dry season and rain fell in torrents nearly every afternoon. While the dry season characteristic of Java as a whole is sometimes more or less evident at Buitenzorg, it never assumes the proportions of a drought. The avenue of talipot palms at Peradeniya and others on the island of Ceylon which flowered in 1918 had been subjected to a prolonged dry season immediately preceding the time of flowering and to several severe droughts during their thirty-eight years of existence. The Corypha at Buitenzorg, on the other hand, had been drenched in rain nearly every day of its life; yet on both islands the palms flowered in the same year.

It would be interesting to know if the flowering of the Buitenzorg talipot commenced in the same month, June, 1918, as did that of the Ceylon palms. One would be inclined in such a case to suspect the presence of some meteorological influence of wide distribution, if one is willing to place any faith in an external stimulus as an influencing factor of even secondary importance. That the palms in Ceylon and at Buitenzorg did flower at very nearly the same time of year is evident from the Peradeniya data and from my observations in Java. (Unfortunately no records are kept of the date of flowering of plants in the Buitenzorg Gardens.) The palms at Peradeniya flowered in June, 1918, fruited during the latter part of 1919 and early in 1920, and died in 1921. The Corypha at Buitenzorg had just dropped its fruit when I first saw it in August, 1920 (fig. 3).

What is true of the talipot palms at Peradeniya and at Buitenzorg is also true of the bamboos at the two gardens. At the time of my stay in Buitenzorg, seven species, out of a total of twenty-four in the Gardens, were in flower. One of these species was *Dendrocalamus giganteus*, which was in heavy flower. The species is the same as that of one of the bamboos which flowered at Peradeniya in 1918. This *D. giganteus* and the six other flowering species of bamboo at Buitenzorg had not been subjected to a drought nor even to a characteristic tropical dry season.

We are, it seems to me, forced to conclude that the ultimate cause of gregarious flowering in bamboos of long life cycle, in particular *Chusquea abietifolia*, *Bambusa arundinacea*, *B. polymorpha*, *Dendrocalamus giganteus*, and *D. strictus*, and in the talipot palm, *Corypha umbraculifera*, is not drought. If drought is at all an influencing factor, then its effect must be relatively slight. While the simultaneous attaining of sexual maturity of three widely differing genera of plants, all of whose life periods are of great length, is an event of such unusual occurrence that one is inclined to wonder if there might not be an external stimulus which is responsible, yet if some such environmental cause does exist we are totally ignorant of what it may be.

The Rhythm in the Sexual Cycle of Bamboos

Evidence of a different kind, which stands in opposition to the assumption that the attaining of sexual maturity of bamboos of long life cycle is

greatly influenced by environmental factors, are the observations made in India which have twice established the cycle of *Bambusa arundinacea* to be exactly thirty-two years. This bamboo flowered in Cisgangetic India (the west coast) in 1804, 1836, and 1868 (18, p. 251). It is of interest to note here that the life cycle of *Chusquea abietifolia*, quite a different type of bamboo, is also about thirty-two years (recorded as thirty-three years) (20).

Less definite, and tending more strongly to support the supposition that the *exact time* of flowering may possibly be somewhat advanced or delayed by external factors, are the interesting data of Kawamura who states that the flowering of *Phyllostachys puberula* has been recorded in old manuscripts of China and Japan as occurring in the following years: 292, 813, 931, 1114, 1247, 1666, 1786, 1848, and 1908 (14). It will be noted that most of the intervals between these dates are either about sixty or multiples of sixty years.

One can, of course, fall back upon the assumption that climatic cycles of rhythmic periodicity also occur and that these determine the regularity of the sexual functioning of bamboos. While certain meteorological phenomena take place rhythmically, *e.g.*, the annual seasonal cycle, it is as yet by no means well established that climatic changes of many years' duration are periodic, and there is little evidence that droughts occur rhythmically.

The evidence so far presented is overwhelmingly against the belief that drought is either the cause of gregarious flowering or that it has any marked influence on reproduction in bamboos. That climate may possibly exert some slight effect on flowering is not, however, to be emphatically denied. While there is little and only indirect evidence in support of this possibility, it is quite conceivable that as a plant of long sexual cycle nears its time of reproduction, unfavorable conditions may hasten the sexual process somewhat. Such a supposition would assist in explaining so remarkable a concurrence of simultaneous flowering as occurred in the Peradeniya Gardens in 1918, though here we must presuppose some climatic factor other than drought.

Depletion of nourishment and injury are two other factors external to the plant which have been advanced as causes of flowering in bamboos. One of these, injury, cannot be regarded as a natural cause of flowering. It has, therefore, no direct bearing on our problem, but it is of interest, and we shall consider it.

Depletion of Nourishment as a Cause of Flowering in Bamboos

The Japanese worker Hori (8) is of the opinion that flowering in bamboos is a "physiological disease." This opinion is in contradiction to that of his fellow countryman Kawamura, who attributes the cause of flowering to the hereditary disposition of bamboos.[5] (The observations of Kawamura

[5] I am indebted to Professor Manabu Miyoshi for calling my attention to work done in Japan on the flowering of bamboos.

will be considered later.) Hori regards flowering in bamboos as a result of an increase in sugar content of the sap due to the inability of the plant to attain the necessary salts for nourishment owing to the dryness of the soil. While Dr. Hori's interesting observations (of which he has kindly given me a *résumé*) tend to support his theory, they cannot be regarded as generally applicable, since, as we have seen, bamboos flower even though profusely watered, and also fail to flower though subjected to repeated seasons of drought.

Macmillan (16) is also of the opinion that a depletion of nourishment is the cause of flowering in some bamboos. He states (p. 125):

> It would thus seem as if the exhaustion of nutriment rather than an infectious influence were responsible for the more or less simultaneous flowering of the Giant Bamboo. The vigorous growth of the plant is such that it cannot go on growing and extending indefinitely. The enormous demands it makes on the soil can be realized by any one who has seen the "ruins" of an old clump, the huge crevices and upheavals formed by the elevated stumps as if the result of an earthquake.

Macmillan's description of the elevated base of an old bamboo clump is very graphic. But on such a mound of stumps measuring fully eight feet in diameter and three feet in height I have seen healthy culms growing as luxuriantly as those of any bamboo clump in the Buitenzorg Gardens.

Macmillan reports the continuation of the vegetative growth of two clumps of *Dendrocalamus giganteus* as a result of increased nourishment. It seems that two of the flowering clumps at Peradeniya, having regained a more vigorous condition, "gave up blossoming entirely, presumably because their circumference had struck richer soil" (16, p. 125).

This instance at Peradeniya is especially interesting because the bamboo in question happens to be of the same species as a young plant recently growing in the Buitenzorg Gardens, which was transplanted from an old clump and thus given an opportunity to regain a more vigorous condition by striking new soil. But it refused the opportunity and soon followed in the path of the parent plant.

There had been growing for many years in the Buitenzorg Gardens a magnificent clump of *Dendrocalamus giganteus* remembered for its size and beauty by all the older workers of the's Lands Plantentuin. In 1918 this entire clump of bamboos flowered and died. Not wishing to lose the last specimen of so fine a bamboo (seeds are not produced), the director of the gardens had a few culms, which were still in healthy condition, removed from the parent clump as soon as the latter commenced to flower. It was hoped that these transplanted culms would continue their vegetative growth without flowering. Such was not the case, however. One of the transplanted clumps soon flowered and died. The second clump lived scarcely more than a year after transplanting, when it too flowered and died. I saw this small plant when the long pendent blooms were still hanging to the then nearly dead culms (fig. 4). New and richer soil did not cause this

Dendrocalamus giganteus to give up flowering and continue its purely vegetative growth. Similar observations must have been made by Brandis, since he writes that "offsets taken from a clump some time before it flowers come into flower at the same time as the parent clump" (1, p. 662).

The hypothesis of depletion of nourishment as the cause of flowering in bamboos could never be applied to those bamboos which flower gregariously. It is quite untenable that each individual of the multitude of plants in a forest of *Dendrocalamus strictus*, one thousand square miles in area in India, or of *Chusquea abietifolia* extending over a region ten miles in length in the mountains of Jamaica, should simultaneously exhaust the supply of food in the soil where they are growing.

Injury as a Cause of the Flowering of Bamboos

Several interesting cases have been reported which support the theory that injury may cause anthesis in bamboos. While injury has no bearing on our problem of the natural cause of gregarious flowering in plants, yet it is worthy of consideration, since it is a probable stimulus which apparently arouses some bamboos to sexual activity.

Gamble states that single clumps of *Bambusa Tulda*, "if badly treated by over cutting or partly uprooted, will afterwards produce flowers without any general flowering" (5, p. 31).

Another instance of the flowering of bamboos being caused by injury is reported by Merrill from the Philippines. In an extensive bamboo forest of Schizostychium one single culm was seen in flower. This culm had been cut by a bolo (machete). The culm was about two thirds severed and in full flower.

Knowledge of these two instances reported from India and the Philippines caused me immediately to suspect that two injured clumps of *Bambusa arundinacea* which I noticed in flower in the Buitenzorg Gardens had also flowered because of the injury received. In each clump several culms were in profuse flower, and these culms were broken off about midway of their length, while all those culms which were not in flower were healthy, uninjured shoots. It seemed possible, therefore, that the broken culms had flowered as a consequence of injury. On second thought it was evident that there was no way of determining without previous data whether the culms had flowered as a result of injury or whether they had broken as a result of flowering. The culms of *Bambusa arundinacea* die after flowering. A dead culm is much less resistant than is a live one to strain from wind, which may be very great on a culm forty to sixty feet in height. In order to ascertain which event, the flowering or the breaking, had preceded the other, I had several culms cut in a large and healthy clump of *Bambusa arundinacea*. These culms when observed one year after cutting had not flowered. The injured culms above mentioned had in all probability broken as a result of flowering and dying and consequent weakening of the culms.

My experiment in cutting these culms was merely to test the effect of injury on this particular species. I subsequently obtained data from India far more convincing. The Chief Conservator of Forests of the Madras Presidency, India, has kindly informed me that in the bamboo-forest areas, many of which are twenty square miles and more in extent, with *Dendrocalamus strictus* and *Bambusa arundinacea* as the predominating growth, the bamboo culms are worked on a rotation of three to four years.

The periodical cutting over and clearing of the individual clumps has had no known effect on the periodicity of flowering.

As for other species of bamboo, there is evidence galore showing how little injury affects the continued vegetational growth of the plants. The most common method of raising bamboos is by cuttings, and so far as I am aware all species lend themselves satisfactorily to this method. The little slender bamboo *Bambusa nana* is commonly used as a hedge plant and is therefore subjected to frequent cutting without any apparent effect on flowering.

Another form of injury which is said to produce anthesis in bamboos is burning. From the Philippines comes the report that, in a clump of Dendrocalamus (species not given) which had been severely injured by fire, the few uninjured or but slightly injured culms had produced flowers. The case was of especial interest because of an observation made by the writer in Jamaica. Fully ninety-eight percent of the plants of *Chusquea abietifolia* seen in the mountains of Jamaica had flowered and died in 1919. Two small patches, however, were found which contained green, healthy plants, and one of these patches had recently been burnt over. The charred stubble was still evident. The parent plants had been burnt to the ground before their life cycle was complete, and the living rootstocks had sent up new shoots which were continuing the growth of the plants and thus carrying on the vegetative portion of the life cycle beyond the normal limit. Burning here not only did not cause flowering, but had, on the contrary, apparently prevented it.

The most convincing example of bamboos flowering as a result of injury that I know of is the report of Branthwaite. He tells of the flowering of three clumps of *Dendrocalamus strictus*. The flowers were borne on short stems which had their origin just below the surface of the ground from the base of culms which had been cut for a clearing on which a hut was built (2, p. 233).

While the sum total of evidence is decidedly against the fact that flowering of bamboos can be induced by injury, the reports of Branthwaite, Merrill, and Gamble suggest that injury may at least sometimes in certain species of bamboos produce anthesis.

The Gregarious Flowering of the Orchid *Dendrobium crumenatum*

Gregarious flowering is characteristic not only of bamboos (and to a limited extent of the talipot palm), but also of the orchid *Dendrobium crumenatum*.

Wherever a number of individuals of the orchid *Dendrobium crumenatum* occur within the same general locality, the plants flower simultaneously. The blossoms of every plant burst forth on the morning and wither in the evening of the same day.

Among the specimens of *Dendrobium crumenatum* in the Buitenzorg Gardens in Java there are plants collected from nearly all parts of the Dutch East Indies, from Riouw (near Singapore), from Sumatra, Java, Borneo, Celebes, and Ambon (a small island at the eastern end of the archipelago). These plants, after being brought to Buitenzorg, *all* flowered on the same day, if they flowered at all. Yet in their native habitat the flowering periods of the plants do not at all coincide. Thus, orchids growing in the virgin mountain forests flower on different days from those in the lowlands. Plants growing at two stations but three kilometers apart may differ in their times of flowering by one or two days. But wherever their original home and whatever the date of flowering there, the plants, when assembled in one locality, flower simultaneously with each other and with the plants which have grown in that locality from youth. There is no other explanation here but that some external factor determines the *exact* time of flowering. The interesting question arises, What is the controlling external factor?

Burkhill, from data obtained in the Straits Settlements, comes to the conclusion that "climatic conditions some eight days in advance of the flowering are a controlling factor" in the gregarious flowering of *Dendrobium crumenatum* (3, p. 405).

The writer has recently (21) published data from Buitenzorg which support the conclusion of Burkhill. If the flowering dates of the orchid are compared, in a table, with the daily precipitation data preceding all the flowering dates, it will be seen that in the majority of instances the rainfall on the eighth day preceding each day of gregarious flowering is unusually heavy. Especially evident does this become when the totals of the precipitation figures for the respective series of days are compared. The total rainfall occurring on the eighth day previous to all the flowering dates is five ninths greater than that of the next highest. The data strongly support the possibility that heavy rainfall eight days in advance of flowering is the cause of simultaneous flowering of the plants. But several striking exceptions occur which force one to conclude that the stimulating factor which arouses the resting flower buds to further activity is not rainfall but some other as yet unknown factor (possibly temperature) commonly associated with heavy rainfall.

At first thought, the gregarious flowering of *Dendrobium crumenatum* is

conclusive evidence that simultaneity of flowering is at least in some plants determined by an external environmental factor. Two striking differences between the gregarious flowering of *Dendrobium crumenatum* and that of bamboos tend further to support this belief. In the bamboos flowering is rhythmic. In the orchid the periods between flowering dates vary from a few days to several months. There is no rhythmic periodicity here.

The second striking difference between the gregarious flowering of orchids and that of bamboos is that in the latter case all the individuals of a bamboo forest are of the same age, while among an assemblage of orchids the individuals may be of quite different ages. Without further consideration one would be inclined to regard some external stimulus as the cause of the irregular gregarious flowering in the pigeon orchid.

The writer has shown, in the article referred to (21), that *simultaneity* of flowering in *Dendrobium crumenatum* rests not upon a climatic but upon a heritable factor, namely, the innate disposition of the plants to develop all their flower buds to the same degree of advancement, at which point growth ceases.[6] The climatic factor arouses the buds—which are all of the same age and which, therefore, all require the same length of time (eight days) to complete development—to further activity, and thus determines merely the *exact time* of flowering and not the simultaneity of it.

Conclusion

As biological science progresses, many vital phenomena, which in the past have been regarded as resident wholly within the organism and in no way determined by the external environment, are one by one shown to be in many instances materially influenced, and in some instances directly determined, by environmental factors. While it must be admitted that one cannot altogether dissociate an organism from its environment, yet this hardly precludes one from regarding some vital phenomena as strictly innate. The origin of variations and mutations forces one, it seems to me, to admit the existence of at least a certain amount of independence of function of the germ plasm from its environment.

The opposition of some biologists to the belief in a hereditary disposition of plants which is responsible for periodicity (in growth, reproduction, etc.) is apparently based on a fear of giving support to any hypothesis which would attribute to a plant self-regulation and would tend to dissociate the plant from its environment. But there is nothing mysterious in periodicity any more than in the radio-activity of certain metals or in the chemical reaction which takes place in a test tube regardless of the surrounding conditions. The causes are merely internal instead of external.

Of the many vital phenomena which are rhythmic in plants—leaf production, leaf fall, cambium activity, reproduction, the synthesis and

[6] For a complete discussion of the possible mechanism involved see the article referred to (21).

solution of starch, etc.—some are undoubtedly susceptible to environmental factors. Furthermore, the same phenomenon varies in different plants in the readiness with which it can be influenced by surrounding conditions. Thus, the winter's rest in some plants is easily altered, in others it can not by any known artificial means be broken. Klebs first clearly showed experimentally that periodicity in plants can be disturbed. In this lies his contribution to biology. But when Klebs would have us believe that *all* the activities of plants are to some extent determined by the outer world, we question his right to do this in the face of his own experiments since he found certain plants which could not be aroused from their winter's rest (15).

But even in those cases in which the normal periodicity can be disturbed, the question arises whether or not the mere ability to alter the normal rhythm of growth by changing the external environment is an indication that this periodicity is actually the direct result of an environmental rather than of a germinal factor. There are some striking instances in which the normal alternation of growth and rest is upset but the plant suffers in consequence. Disturbing the usual growth rhythm results in weakening the plant's vitality. An excellent illustration of this exists at Tjibodas, Java, where there is a small apple tree growing in the acclimatization garden in the mountains. This temperate-zone tree has been growing in the tropics for some twelve years or more, in a climate which has no pronounced seasonal change. Its normal periodicity of growth and rest has been disturbed but *not done away with.* The tree is undersized (four feet high) and has never borne fruit. It stands there an unhappy specimen, with, when I saw it, one branch in full foliage, another without any leaves at all, and still another with well-developed buds. It seems to be having a sad time trying to exist in a seasonless climate with an inherent periodicity of growth and rest manifesting itself at different times of the year on different branches. The periodicity is there. The seasonal cycle of temperate regions would have determined *when* the rest and growth periods should occur. In a tropical climate this seasonal guidance is lacking and the normal rhythm of growth and rest is disarranged, but the inherent periodicity is still evident.

The fact that the winter's rest in plants can in many cases be disturbed has led other workers than Klebs to come to rather far-reaching conclusions. Thus Howard, as a result of some very extensive work on the treatment of dormant woody plants for forcing them into growth, concludes that "all of these forms of rest are caused by unfavorable external conditions" (9, p. 5).

Just what the unfavorable conditions are which cause all kapok trees (*Ceiba pentendra*) *simultaneously* to become completely defoliated each year at Buitenzorg, where there is no pronounced seasonal change, it is difficult to see. Even more striking is the case of Ceiba (*C. occidentalis* ?, the silk-cotton tree) in Jamaica which annually loses all its leaves, but not simul-

taneously with other silk-cotton trees. Why do the "unfavorable external conditions" which cause one silk-cotton tree in Jamaica to rest from leaf production not likewise cause another silk-cotton tree standing near by to rest also?

Howard's further deduction, that "a plant readily adapts itself to the new demands and the rest becomes a habit," is perhaps applicable to some plants, but not to all. The tropical palm has not yet adapted itself to a temperate climate and acquired the habit of resting in the winter; nor has the temperate-zone apple tree at Tjibodas very successfully adapted itself to a tropical climate after twelve years or more of existence there, even though the climate at Tjibodas is not unlike a temperate summer as regards moisture, temperature, and light.

One fundamental objection to the belief in a heritable periodicity in bamboos has been raised by several writers. It is pointed out as a remarkable fact that "not only mature clumps but quite slender seedlings" (11, p. 126), "even the buds which have just appeared out of the ground" all blossom at the same time (19, p. 6). This brings us to the consideration of a rather theoretical question, What constitutes age?

That the parent culms in a large bamboo forest of Dendrocalamus or Chusquea are all of the same age is self-evident. They all sprang from seed sown at the same time, *i.e.*, at the time of the simultaneous death of the individuals of the previous forest. As for the "young" shoots, their age is, from one viewpoint, the same as that of their parents since they arose from the same rootstock. Meristematic cells, in the cambium ring, for example, remain perpetually young, though in years they are older than most of the cells which make up the tree. Old cells become young when lateral shoots are formed from old wood in trees, or when in lower animals limbs are regenerated. If we grant that the morphology and function of cells is dependent on their location in the plant, that is, that there is no such thing as specificity of cells, then all the cells of a bamboo clump, in "young" shoots as well as in "old" culms, are potentially the same; therefore, all are alike affected by age. Consequently, the determiner present in the germ plasm of the "old" culms which causes them to reach sexual maturity at a definite time is likewise present in the "young" culms which arise from a common rootstock.

How this innate sexual periodicity of some bamboos came into existence it is impossible to say. Either it must have been established in the past as an acquired habit, or it must be purely the expression of the original physical and chemical make-up of the germ plasm. The nicety with which the life cycle of annuals and the growth rhythm of perennials fit in with the seasonal changes of temperate regions leads one to believe that these periodic vital phenomena have been induced through the ages by climatic conditions, with the result that the periodicity has become innate, the habit being more firmly established in some plants than in others. The same

may be true of bamboos of long life cycle, although in this case the climatic factor is apparently no longer active.

The belief in a germinal factor as the cause of gregarious flowering in bamboos does not imply that this heritable determiner is past being influenced by the external environment. The finding of green specimens of *Chusquea abietifolia* in the mountains of Jamaica when fully ninety-eight percent of the total number of plants were dead suggests that the usual periodicity of this bamboo has in some individuals become altered. As was pointed out in the introduction of this article, it is the task of the biologist to ascertain to what extent this or that character is susceptible to external influence, *i.e.*, to ascertain the degree of fixity of the innate factor. If it is found that a vital process cannot be altered, then we must admit either that it is too firmly established in the germ plasm to be disturbed, or else that we have not found the requisite environmental factor. This latter assumption is made by Klebs.

It is impossible to deny the assumption of Klebs that where we are unable to find the controlling environmental factors we have simply failed to search far enough; yet, until the exact combination of external stimuli is found, the theory that gregarious flowering is determined by a germinal factor stands without disproof.[7]

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[7] After this article was written there appeared an account by Wieland (Amer. Jour. Bot. **8**: 218-230. 1921) of monocarpy in the cycadeoids. There is evidence that at least two species of fossil cycads flowered but once in a lifetime. Four other species show a tendency toward monocarpy. The most convincing record is that of a specimen of *Cycadeoidea Dartoni*, the armor of which is packed with hundreds of mature cones. Ample sections of this specimen show no trace of a succeeding foliar crown; although in the very different species, *C. ingens*, a fine crown of young fronds surmounts a scattered growth of flower buds. It is, therefore, reasonably evident that *Cycadeoidea Dartoni* was monocarpic.

It is most interesting to have so authentic a record of monocarpy among the gymnosperms of past geologic ages.

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EXPLANATION OF PLATE XII

FIG. 1. The avenue of talipot palms, *Corypha umbraculifera*, in the Peradeniya Gardens, Ceylon, in December, 1920. Seven of the sixteen palms forming the avenue flowered in June, 1918. One of the palms which had flowered stands in the front on the right side of the picture. The fruit has fallen, leaving only the bare stalks of the inflorescence.

FIG. 2. Another Corypha in the Peradeniya Gardens which flowered some years before those shown in figure 1, and which, unlike those palms, received for at least four years previous to the time of flowering an annual rainfall above the average normal. This picture gives some idea of the luxuriance of the inflorescence. (The photograph is published through the courtesy of Plâté, Ltd., Ceylon.)

FIG. 3. A telephotograph of the crown of a *Corypha umbraculifera*, after fruiting, in the Buitenzorg Gardens. This talipot palm had never experienced a drought; indeed, it was drenched in rain nearly every afternoon of its life, while those palms shown in figures 1 and 2 were annually subjected to a dry season which frequently assumed the proportions of a drought. Some few of the leaves of the former crown of foliage are still to be seen clinging to the trunk. The palm is dead, or nearly so.

FIG. 4. A small clump of *Dendrocalamus giganteus* in full flower in the Buitenzorg Gardens. Long pendent inflorescences are abundant, while but few leaves remain on the now nearly dead culms. These bamboo shoots were taken from an old clump of *D. giganteus* which was beginning to flower. The "young" transplanted culms flowered soon after the parent plant.

SEIFRIZ: CAUSES OF GREGARIOUS FLOWERING

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NOTE ON AN INDIRECT EFFECT OF SPRAYING POTATOES WITH BORDEAUX MIXTURE

J. T. ROSA, JR.

(Received for publication January 16, 1922)

Application of copper sprays to Irish potatoes has long been advocated for the control of the early and late blights, as well as for a supposed physiological effect of copper on the plants, whereby they are kept in a green, growing condition for a longer time than unsprayed plants. More recently attention has been called to the use of Bordeaux mixture for the control of tip-burn, by Erwin (3), and for the prevention of hopper-burn, by Dudley and Wilson (1). However, a spraying experiment on the horticultural grounds at Columbia, Missouri, conducted during the spring and summer of 1921, indicated that, under certain conditions, the application of Bordeaux or other spray having similar physiological effects may produce indirect results which are undesirable.

The variety used was Early Ohio, a good strain of which was obtained from northern Michigan and planted March 17, under uniform conditions. Four applications of 4–4–50 Bordeaux were made during May and June, both with and without arsenate of lead and nicotine sulphate. The sprayed plants remained green about three weeks longer (see fig. 1), and yielded an average of 34.2 percent more, than the checks, when dug August 28th. Since neither early nor late blight was present, but leafhoppers were numerous as usual after July 1, these results may be ascribed to tip-burn and hopper-burn control, and perhaps to other physiological effects. However, the tubers from the sprayed plots consisted to a large extent of knobby second growths, so that the actual quantity of marketable potatoes was really much less than from the check plots, the tubers of which showed second growth only to a moderate extent (see fig. 2).

The Early Ohio, which is the leading variety at present in the lower Corn Belt, seems much more subject to second growths of the tubers than other varieties grown at Columbia the past four years. Perhaps such varieties as the Irish Cobbler, which has little tendency to second growth, would react quite differently if subjected to spraying treatment under the same conditions as those mentioned in the test with the Early Ohio variety. If the increase in yield can be secured, without distortion of the tubers,

[The **Journal** for February (**10** : 57–112) was issued March 2, 1923.]

on satisfactory early sorts such as the Irish Cobbler, spraying should be a profitable practice in this region.

FIG. 1. Effect of Bordeaux mixture on vitality of Irish potato plants. 2 rows on right unsprayed. On left, sprayed four times with Bordeaux mixture 4–4–50 alone, also the same with arsenate of lead, and with arsenate of lead plus nicotine sulphate. Photographed at Columbia, Missouri, July 25, 1921.

During the growing season the past year, the weather alternated, with short wet periods interspersed between longer hot, dry spells. The result, of course, was a sharp variation in soil moisture in the potato field. It occurred to the writer that such soil-moisture fluctuations might be the immediate cause of the second growth on the potato tubers, especially on those from the sprayed plots, which continued in vigorous growing condition during that portion of the summer when the soil-moisture fluctuations were sharpest. Furthermore, since Duggar and Bonns (2) have shown that a film of Bordeaux spray on the leaves of the potato plant increases the transpiration rate, it may be supposed that the water deficit is greater in sprayed plants in the field during dry periods. This notion appears all the more probable when the larger expanse of leaf surface possessed by the sprayed plants is considered. Presumably, tuber growth ceases when lack of moisture makes conditions unfavorable for the development

of the plant as a whole. When rain comes, growth again proceeds vigorously for a few days. The tuber development resulting from such spurts of growth, in the case of the Early Ohio variety, seems to consist very largely in knobby protuberances from the eyes, especially at the tips of

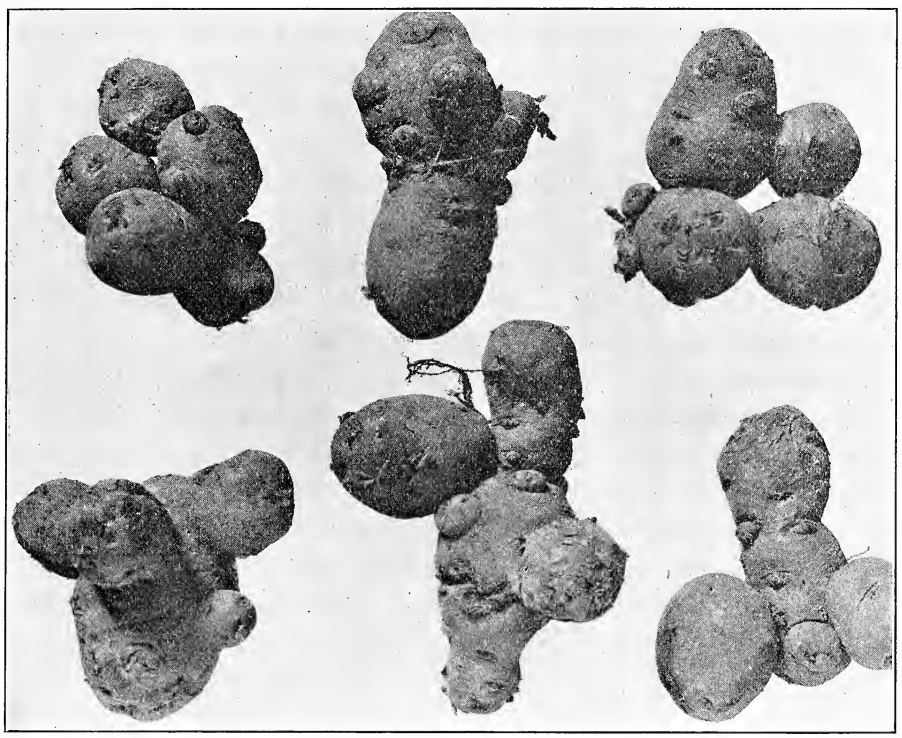

FIG. 2. Knotty protuberances or second growths on tubers of early Ohio potatoes from plots sprayed four times with Bordeaux mixture.

tubers previously formed. As many as four or five distinct growth zones could be identified on some of the knotty tubers from the sprayed plots. It is hoped that further experiments can be carried out to learn the limits of variation in soil moisture which the potato will endure without developing second growths, as well as the relation of certain other edaphic factors to tuberization. The state of dormancy in tubers whose growth is checked as above indicated is also a question of some interest, for if removed from the plant these tubers would not commence stem growth for many weeks.

Another interesting question suggested by the observations described above is the value of tubers bearing second growths for seed purposes. If variation in soil moisture or in some other environmental factor causes tubers to develop second growth in Missouri, this defect may arise from the same causes in Wisconsin, Minnesota, or other seed-growing section. Then tubers bearing second growth would not necessarily be weak or de-

fective in any way physiologically, and there need be no discrimination against such tubers for seed purposes, although such discrimination has been customary. In fact, the experiments of Dr. Salaman (4) in England showed that knobby second growths planted as seed-pieces produced a larger crop than pieces from normal tubers, and that the tendency to second growth was not transmitted. This suggests another question, then, as to the advisability of submitting to critical tests some current ideas concerning standards of quality in Irish potatoes, at least from the standpoint of seed growing and plant improvement.

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INTERNAL DECLINE OF LEMONS

II. GROWTH RATE, WATER CONTENT, AND ACIDITY OF LEMONS AT DIFFERENT STAGES OF MATURITY [1]

E. T. Bartholomew

(Received for publication April 12, 1922)

This work was undertaken to determine the possible bearing on the etiology of internal decline of lemons [2] of (*a*) the rate at which lemons increase in size as influenced by climatic and seasonal changes and by the time of year at which the fruit is set, and (*b*) the increase in acidity and water content of the fruits at different stages in their development.

Statements have been made by different writers concerning the acidity and water content of mature lemons but not of lemons at different stages of maturity. Wehmer (8) gives the water content of mature lemons as 82.64 percent, a figure which is too low, at least for California lemons, as is indicated later in this discussion. Clark and Lubs (2) give a pH value of 2.2 for the true acidity of juice from mature lemons. The results obtained in this work agree very well with that figure.

Methods of Experimentation

Groves located at Corona, Upland, and Riverside were chosen as suitable places in which to carry on the work. These groves are all of different ages, as follows: at Riverside, 6 years; at Corona, 20 years; and at Upland, 30 years. All three groves are Eurekas, the variety usually grown in California.

The sizes of the lemons were determined by diameter measurements with a vernier caliper. In each of the three groves 200 small lemons were measured and tagged each month for one year. When a new lot was measured and tagged, those previously tagged and measured were remeasured, so that each lemon was measured each month until it dropped from the tree or until it came to maturity and was picked. Fruits were tagged on a chosen group of trees and in many different locations on each tree. Fruits averaging from 0.44 to 1.29 cm. in diameter were chosen for the initial measuring and tagging. Fruits of these sizes are about 1 to 2 months old, depending upon the season and other conditions attending their growth. Each month, following the month of initial measuring and tagging, samples were brought to the laboratory and tested for acidity and water content.

[1] Paper no. 95, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] For a description of the abnormality known as internal decline of lemons, the reader is referred to the first article in this series by Bartholomew, Barrett, and Fawcett (1).

In making the tests for acidity and water content, the lemons were cut once longitudinally and once transversely, thus dividing them as nearly as possible into 4 equal parts. One half of each end was used for the acidity test and the other half to determine the water content. This was done to determine whether or not there were any differences between the acidities and water contents of the two ends of the lemon. In determining the acidity the fleshy pulp alone of the fruit was used, but both pulp and peel were used in determining the water content.

The hydrogen electrode was used to determine the acidity. The pulp to be tested was cut free from the peel and ground in a mortar, and the juice was extracted in a tincture press. In making the water-content determinations the peel and pulp were finely cut, placed in weighing bottles, and dried to constant weight in a vacuum oven.

In connection with the determinations of acidity of lemons at different stages of growth, other tests were made to determine the acidity of mature lemons taken from the packing houses or directly from the trees. A series of experiments also was performed to determine whether or not the leaves of the lemon trees may be an active factor in drawing water from the fruits.

Results and Discussion

Growth Rate and Water Content of Lemons

The subjects of growth rate and water content are so closely related that no attempt will be made to discuss them separately.

The Eureka lemon tree is one which, under conditions of comparatively high humidity and low temperature, such as prevail near the coast in southern California, produces new fruit in practically every month of the year. Farther inland, where the humidity is lower and the temperature higher, there is a more marked tendency toward seasonal production. In this respect, fruit production apparently is influenced also by the age of the tree, because the older the tree the greater tendency it shows toward seasonal rather than continuous production. Evidence of this latter condition was shown in the three groves chosen in which to carry on this work. It was found that no fruits were set during the month of September at Riverside where the trees were 6 years old; none during September and March at Corona where the trees were 20 years old; and none during August, September, October, and March at Upland where the trees were 30 years old.

It was found also that, although there is a tendency toward continuous production of fruits, many more are produced during the spring set of fruit than at any other time of the year. This agrees with the findings of Reed (6) in his study of the relation between the flowers and the fruits of the lemon. He found that approximately 66 percent of the fruit buds appear during March and April, 17 percent from May to October inclu-

sive, 13 percent in November, and about 3 percent from December to February inclusive.

The month of the year in which fruits are set determines to a certain extent whether they will soon drop off or will remain on the tree till they mature. The results of the experiment as carried on in these three groves indicate that, at least under climatic conditions such as prevailed in this region from June 9, 1920, to August 9, 1921, fruits set in April, May, and June have the best chance of reaching maturity. Climatic and soil conditions and the time of year at which fruits are set are important factors in determining whether or not they will remain on the trees till mature. This is shown by the fact that of some of the lots of 200 lemons tagged, practically 100 percent remained on the trees, while of some of the other lots as much as 50 or 60 percent, and in one or two cases even 90 percent, fell off before they were more than 2 months old. That the mortality is often very high is indicated perhaps more strikingly by Reed's (6) work, which shows that out of 4,440 flower buds, 51.98 percent set fruits, 21.71 percent reached a diameter of ¼ inch, and only 6.62 percent reached maturity.

It was found that the growth rate of individual lemons is influenced not only by soil and climatic conditions and the time of the year when set, but also by the location on the tree. While the height on the tree and the location, whether within the foliage or exposed on the outside, appeared to have an influence, the most important factor seemed to be the condition of the branch on which the fruit was borne. While this is true also for deciduous fruits, it appears to be more marked in the case of lemons. This variation is probably due to differences in the amounts of water and food substances carried by the different branches. It was found, for example, that some of the fruits set in April were ready to be picked in the following October and November, a period of 7 and 8 months after being set, while other lemons on the same tree were not up to picking size for at least 14 months after being set.

It is impossible to present here all the interesting data obtained in these experiments on growth rate, water content, and acidity of lemons. The figures given in table 1 are a summary of the data obtained in one of the series in the grove at Corona. This is a fair example of all the data secured in all the series. The lemons in this series were first measured and tagged August 8, 1920. The average diameter when tagged was 1.15 cm.

It will be seen by reference to table 1 that the growth rate of the lemons was fairly constant from August 8 to February 10, during which period the lemons increased in size from 1.15 to 4.60 cm. However, on the date of the next measurement, March 9, the average size of the lemons had decreased from 4.60 to 4.53 cm. Further observation will show that another slight decrease took place from June 9 to July 8. A glance at the figures in the columns headed "water content" will show that at the time of this last

decrease in size of the lemons there was a corresponding decrease in the water content. While there was not an actual decrease in water content from February 10 to March 9, yet the increase was very small, far from commensurate with previous increases.

TABLE 1. *Growth rate, water content, acidity, and color of lemons at different stages of maturity*

Date, 1920 and 1921	Average Diameter (cm.)	Percent, Each Color			pH Value		Water Content (percent)	
		Green	Silver	Yellow	Stylar End	Stem End	Stylar End	Stem End
Sept. 10.....	2.17	100			4.46	4.46	53.97	53.68
Oct. 9.....	2.86	100			2.91	3.08	75.42	74.50
Nov. 10.....	3.34	100			2.64	2.71	81.45	81.61
Dec. 10.....	3.63	100			2.54	2.50	83.03	82.16
Jan. 10.....	3.91	68.1	31.9		2.60	2.64	84.01	84.21
Feb. 10.....	4.60	18.9	75.8	5.3	2.57	2.54	85.57	85.69
Mar. 9.....	4.53	11.4	64.5	24.1	2.33	2.36	85.74	85.93
Apr. 11.....	4.73	6.8	71.6	21.6	2.30	2.33	86.73	86.72
May 9.....	4.92	4.8	76.2	19.0	2.23	2.27	88.99	88.59
June 9.....	5.18	One Lemon	73.0	25.1	2.29	2.33	89.99	88.82
July 8.....	5.16	One Lemon	71.2	26.4	2.29	2.33	88.20	87.30
Average					2.65	2.69	82.10	81.75

The facts that on the dates of two measurements, March 9 and July 8, the lemons were actually smaller than at the time of the preceding measurements, and that these decreases in size were accompanied on March 9 by only a very slight increase in water content and on July 8 by an actual decrease, raise the interesting question as to what may have been the cause of these conditions. The results of the following experiments give a direct answer to this question.

1. Lemon branches about 45 cm. long, each bearing from 1 to 3 mature or nearly mature lemons, were brought into the laboratory. The lemons were detached from half of the branches while the remaining branches were left as brought from the grove. The detached lemons, the branches from which they had been taken, and the branches bearing lemons were all placed on the laboratory table. At the end of 24 hours it was found that the detached lemons, as nearly as could be detected by the touch, were firm and in normal condition while the leaves on the branches from which these lemons had been taken were wilted and drooping. On the other hand, the leaves on the branches to which the lemons were still attached retained their luster and were normally rigid and upright while the lemons on these branches had become quite soft to the touch.

Further experimentation showed that the leaves on branches from which the lemons had been detached showed signs of wilting within a very few

hours, while the leaves on branches to which the lemons were still attached remained turgid and upright for thirty-five hours or more. The length of time depended, of course, upon the number of leaves per fruit and the humidity and temperature of the surrounding air.

2. Small branches, each bearing about 8 or 10 leaves and one lemon, were chosen for the next experiment. A thin slice was cut from the stylar end of each lemon and the cut end was immersed in eosin. The remainder of the lemon and the branch were left exposed to the air. Examinations were made at intervals. It was found that by the end of 12 to 15 hours the pull of evaporation had drawn the eosin up through the lemon and out into all the leaves.

3. Experiments were performed to determine the comparative amounts of water lost by detached lemons as compared with those remaining attached to the severed branches. No attempt was made to control the temperature or humidity conditions. Determinations were made after the detached lemons and the lemons still remaining attached to the branches had remained under ordinary laboratory conditions for certain lengths of time. At the expiration of a given time the entire lemons were cut fine, placed in weighing bottles, and dried in a vacuum oven. The results showed that for any given time the lemons that were still attached to the branches lost 12 to 15 times as much water as those which had been detached from the branches. Experiments similar to numbers 1, 2, and 3 were performed by Hodgson (4) on the navel orange with the same general results.

4. The fact that lemons allowed to remain attached to branches cut from the tree will lose water much more rapidly than lemons detached from the branches was further proved by the freezing-point method. Two branches were taken from each of 10 trees. These branches were divided into 2 lots in such a manner that each lot contained one branch from each tree. Each branch bore one lemon about 4.45 cm. in diameter. The lemons from one set of branches were at once detached and prepared for making the freezing-point determination. The other set of branches, with the lemons still attached, was placed in an oven at 46° C. for 4½ hours. At the end of this time these lemons were taken from the branches and treated as in the case of the first lot. In preparing the lemons for the freezing-point test one third of each end of each lemon was used. Both peel and pulp were ground in a meat grinder, and the juice was extracted in a tincture press. In order to get a check on this method of treatment the experiment was repeated in all details except that the second lot of lemons was placed in the oven for 4½ hours at 46° C. after they had been detached from the branches. An average of ten lemons was used in each trial.

A summary of the results of this experiment is given in table 2.

TABLE 2. *Comparative losses of water from attached and detached lemons as shown by the freezing-point method*

No. of Test	Nature of Tests		Mean Δ	Mean Concentrations (atmospheres)
1	Lemons detached from branches at once..	Stylar end.	1.077	12.96
		Stem end..	1.062	12.79
	Lemons detached from branches *after* being in oven 4½ hours at 46° C.....	Stylar end.	1.193	14.35
		Stem end..	1.198	14.37
2	Lemons detached from branches at once..	Stylar end.	1.182	14.22
		Stem end..	1.150	13.84
	Lemons detached from branches *before*.. being placed in oven 4½ hours at 46° C...	Stylar end.	1.192	14.35
		Stem end..	1.196	14.39

The results in the first half of table 2 show that there was a marked increase in the concentration of the juice in the lemons that were in the oven for 4½ hours, the increase being 1.39 atmospheres for the stylar end and 1.58 atmospheres for the stem end. That this increase in concentration could not have been due, to any marked extent, to water passing out through the peel of the lemons is shown by the figures in the latter half of the table, for here the stylar end shows a difference of only 0.13 atmosphere and the stem end one of only 0.55 atmosphere. The marked difference between the first and second tests is due to the fact that the first was made about 6 weeks earlier than the second one. At the time of making the earlier test the trees were still growing, and the determinations were made after irrigation and a light rain followed by heavy dews at night. This, of course, would tend to make the fruits more turgid, and hence to reduce the sap concentration.

5. The results of another experiment are offered as further proof that the leaves may draw water from the fruits. Branches about 60 cm. long, each bearing from 1 to 3 lemons, were cut from the trees and at once cut again under water. These branches were then brought to the laboratory, placed with the cut ends in jars of water, and all set in a well-ventilated oven heated by electricity. During the day the oven temperature ranged from 35 to 38° C. About 5 p.m. the heat was turned off and not turned on again until about 8:30 a.m. the next day. The temperatures at night ranged from about 15 to 18° C. The leaves on the branches under these conditions retained a normal appearance for about 5 to 7 days. At the end of this time they began to wilt and lose their luster. By the end of 8 to 10 days the leaves began to drop. About this time the lemons were taken from the branches and thin slices were cut from the stylar ends, the knife passing through the point of juncture of pulp and peel. Observation of the cut surfaces showed that in a large number of the lemons, though not

in all, the tissue had broken down, thus leaving small circular openings about 1 mm. in diameter adjacent to each main vascular bundle. These openings have been termed "drying-out holes." Cutting of control lemons at the time these branches were taken from the tree showed that these lemons were in a normal condition at the beginning of the experiment. Thousands of lemons taken from dry portions of groves and cut have shown these drying-out holes, while comparatively few or none of the lemons from the more moist portions of the same groves showed them. The variation in moisture content in different parts of a grove may be due to such factors as differences in soil texture or an uneven distribution of irrigation water.

That there must be some relation between these drying-out holes and the appearance of internal decline is indicated by the fact that practically every lemon having decline shows these holes when it is cut for examination. This experiment coupled with the field observations indicates that this collapse of tissue, caused by the withdrawal of water from the fruit, is the first visible step in the production of internal decline.

The results of the preceding 5 experiments show very clearly that the lemon fruits act as water reservoirs for the leaves. When the roots fail to supply adequately the demand of the leaves for water, the leaves begin to draw it from the fruits. In a climate such as exists in the inland lemon-growing districts of California, the leaves will begin to draw water from the fruits even before the soil moisture has become very materially depleted. On going into a grove in the afternoon on a warm summer day when the temperature is near 100° F. and the psychrometer shows a humidity of 20 percent or less, the lemon fruits are found soft to the touch even though the soil moisture content may be well above the wilting coefficient *of the leaves*. Upon examination of the same lemons the following morning they will be found turgid. This condition becomes especially noticeable when there is a wind blowing, since this increases the rate of evaporation from the leaves. The withdrawal of water from the fruits by the leaves is an important factor in irrigated districts such as this, because, if the water supply in the soil becomes too low, or if climatic conditions remain unfavorable too long, not only the fruits but also the leaves may be caused to fall from the trees.

In connection with the foregoing statements it might be well to emphasize the fact that, at least in the case of such plants as citrus trees, which may retain their fruits from several months to a year or more, the term "wilting coefficient" of the soil, as usually applied, has little significance. There can be no doubt that water may be withdrawn from the fruit to an injurious extent by the leaves long before there is any sign of wilting in the leaves. For such plants as the citrus fruits it would be interesting and profitable to have determined a wilting coefficient based on fruit rather than on leaf conditions. However, this would probably be difficult except as a coefficient might be worked out for each individual kind of fruit.

These experiments proving that water may be withdrawn from the fruits by the leaves are not given as proof of a new discovery. They are reported (*a*) because it appears that the importance of this phenomenon of water-withdrawal from the fruits by the leaves is not generally realized, (*b*) because of their bearing on the major experiments reported in this article, and (*c*) because of their bearing on our practice of growing citrus and similar plants under irrigation in a semi-arid region, rather than in their native habitats which have abundant rainfall and high humidity. Further discussions bearing directly or indirectly on this subject of withdrawal of water from the fruits by the leaves may be found in articles written by Renner (7), Livingston and Brown (5), Coit and Hodgson (3), Hodgson (4), and others.

By referring to table 1 it will be seen further that there is very little difference between the water contents of the two ends of the lemon. This series shows an average of 0.35 percent more water in the stylar end than in the stem end. Some of the other series show even less difference than this series.

It was found during these experiments that the water content of young fruits is affected by the availability of water. For example, in September, when the water supply was limited and relative evaporation was high, the water content of lemons 1.9 cm. in diameter averaged 54 percent. In December, when both rain and irrigation water were available and the amount of evaporation was comparatively small, the water content of lemons only 1.27 cm. in diameter averaged 68 percent. The variation of water content as affected by availability of water and climatic conditions was noticed also in the mature lemons. The variation may be as great as 10 percent or more, but it is usually less. The range of differences of water contents in the mature fruits tested from the three experimental groves was from 88.20 to 92.14 percent. Wehmer (8) reports only 82.64 percent for mature lemons.

Further reference to table 1 will show that the increase in water content is comparatively small after the lemon has attained an average diameter of about 3.8 cm.

During the course of these experiments it was noted that there were two periods in the year when there was a tendency toward cessation of growth. One occurred during the colder months and probably was due to climatic conditions that caused a decrease in metabolic activity and a tendency toward a normal rest period. The other occurred during the hottest months of the year and in all probability was due to low humidity and insufficient soil moisture. It is quite often the case in lemons that are approaching maturity that, when growth begins again after being checked, it occurs almost wholly in the peel, thus making a lemon with a thick peel which is undesirable for marketing.

Acidity

It was found in studying the acidity of the lemons that, while the total acid content increases as the size of the lemon increases, the true acidity of the juice, like the water content, increases comparatively little after the lemon has reached a diameter of about 3.8 cm. By referring to table 1 it will be seen that in this series, lemons averaging 3.63 cm. in diameter showed average pH values of 2.54 for the stylar end and 2.50 for the stem end, while 5 months later, when the lemons in this same series had attained an average diameter of 5.18 cm., the pH values were only 2.29 and 2.33. In another series, when the average diameter was 4.32 cm., the pH values were 2.57 and 2.54. Eight months later the average diameter had increased to 5.72 cm., but the acidities had increased only to pH 2.33 and 2.36. These two examples are typical of all the series.

Lemons which are all approximately of the same age and size show a great deal of individual variation in acidity. For example, mature lemons taken from storage and tested individually showed variations in acidity such as the following: pH 2.27, 2.40, 2.37, 2.29.

The average acidity obtained for all the mature lemons tested was pH 2.31. This value is somewhat lower than it would have been had the tests been made on first-class market lemons. Some of the lemons tested in this work had been allowed to remain on the trees until they were in the condition known to the growers and packers as tree-ripe.[3] When lemons have reached this condition they have a slightly higher sugar and slightly lower acid content than earlier.

Variations in acidity were found not only in individual lemons but in the different ends of the same lemons. In some lemons it was the stylar end and in others the stem end which showed the higher acidity. This may be seen in table 1, and in the figures given in the second paragraph preceding this one. However, the tests, when averaged, showed practically no difference between the acidities of the stylar and stem ends of over 400 normal lemons. The average of the total number of tests showed the stem end to be pH 0.01 more acid than the stylar end. This difference is so small, however, that it comes well within the limits of experimental error.

Summary

The principal results obtained by the experiments on growth rate, water content, and acidity of lemons at different stages of maturity may be summarized as follows:

1. While the lemon tree tends toward the production of new fruits continuously, the age of the tree and climatic and soil conditions make the production more or less seasonal. In the inland districts the seasonal setting of new fruits is more marked than in the coastal regions.

[3] For an explanation of the term "tree-ripe" and similar terms referring to the stages of maturity of lemons, see the first article in this series by Bartholomew, Barrett, and Fawcett (1).

2. The time of the year when set, the age of the tree, and climatic and soil conditions are all factors determining the growth rate of the fruits. Some fruits may mature in 7 or 8 months, while others growing on the same tree may require as much as 14 months in which to mature.

3. Lemons may actually decrease in size while still attached to the tree, in consequence of the withdrawal of water from them by the leaves. This withdrawal of water from the fruits by the leaves may result in the collapse of at least a portion of the tissue in the stylar end of the fruit.

4. The wilting coefficient of the soil as indicated by lemon leaves can not be considered a safe criterion as to whether or not the lemon fruits are suffering from a lack of water.

5. There is practically no difference between the water contents of the two ends of the normal lemon.

6. As the lemon enlarges, its water content increases, but this increase is much more rapid up to the time that it is about 3.8 cm. in diameter than from that time to maturity.

7. The size of the lemon is not necessarily proportional to the percentage of water it contains. In September a lemon 1.90 cm. in diameter may have a much lower water content than a lemon 1.27 cm. in diameter in December.

8. Mature lemons may show considerable variation in water content. The range in this series of experiments was from 88.20 to 92.14 percent.

9. While the total acid content of the lemon increases rapidly as it approaches maturity, the true acidity increases very little after the lemon has reached a diameter of about 3.8 cm.

10. There are quite wide variations, but the average of a large number of stylar and stem ends of normal lemons shows the mean acidity to be substantially the same for each.

11. Mature lemons of practically the same age and size have a comparatively wide range of acidity.

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INFLUENCE OF TEMPERATURE ON THE PECTINASE PRODUCTION OF DIFFERENT SPECIES OF RHIZOPUS

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Investigations (1) have shown that the following species of Rhizopus with the exception of the last two are parasitic on the sweet potato: *nigricans* Ehrnb., *reflexus* Bainier, *tritici* Saito, *artocarpi* Racib., *delemar* (Boid.) Wehmer and Hanzawa, *maydis* Bruderl., *nodosus* Namysl., *oryzae* Went and Pr. Geerligs, *arrhizus* Fischer, *microsporus* v. Tieg., and *chinensis* Saito. These fungi produce an enzym which dissolves out the middle lamellae so that the cells lose their coherence, thereby transforming the potatoes into a soft, watery mass. The cells themselves, however, are not penetrated, at least in the early stages of decay. It was likewise shown (2) that the two nonparasitic as well as the parasitic species produce an enzym, a part of which is exuded into the substrate which macerates raw sweet-potato disks in from two to four hours. The nonparasitic species actually produce more enzym than some of those which cause decay of sweet potatoes. Furthermore, it was found that the maximum strength of the macerating enzym both in the solution and in the mycelium is reached after a two or three days' growth of the organisms in sweet-potato decoction.

Harter, Weimer, and Lauritzen (1) showed that these species of Rhizopus could be roughly grouped into high-, medium-, and low-temperature forms. The results demonstrated that a low temperature is not so much a limiting factor to infection and decay as a high temperature, since intermediate forms produced decay under laboratory conditions at relatively low temperatures, while the low-temperature forms did not infect sweet potatoes at a temperature of 30° C. or above.

In view of the relationship found to exist between the different species with respect to the temperature at which they will infect and decay sweet potatoes, the writers proposed to determine (1) if pectinase is produced at any temperature at which the fungus will grow, and (2) if the amount produced is greatest at the optimum temperature for infection and decay.

Method of Experimentation

The methods employed are, for the most part, the same as those previously described by the writers (3), but modified when necessary to meet the requirements of the problem. The fungi were grown on sweet-potato decoction in 2-liter Erlenmeyer flasks held in incubators maintained at constant temperatures of 9°, 20°, 30°, and 40° C. The organisms were

grown at the three higher temperatures for 3 days and at the lower for 26 days. A luxuriant growth resulted in 3 days at 20°, 30°, and 40° C., thereby furnishing a sufficient amount of mycelium for enzymic studies. However, at 9° barely enough material for this purpose was produced after 26 days of growth. The decoction in the two flasks upon which the same species had grown was combined into one compound sample, and aliquot parts were taken for maceration experiments. The solution was handled and the mycelium treated according to a method previously described (3). Maceration was regarded as complete when the disks offered no resistance when pulled from opposite directions. The enzymic action of the mycelium was determined by suspending 0.50 g. of pulverized material in 25 cc. of distilled water, in accordance with methods previously described (3).

The raw disks (1.5 mm. in diameter and 0.5 mm. thick) which were employed to measure the macerating power of the enzym in each experiment were cut from within the fibro-vascular ring of one sweet potato. The experiments with all the organisms were repeated several times at each temperature. Flasks in which the enzym was inactivated by steaming, as well as flasks containing some of the original decoction which had not been inoculated, were employed as controls in all the experiments. The sweet-potato decoction was made in sufficient quantity of uniform composition for one entire experiment with all the species.

Maceration was carried out at 40° C. regardless of the temperature at which the organisms were grown. The solutions, previously filtered through cotton to remove the fungous material, were brought to the temperature at which maceration was to take place by exposing them for one hour at 40° C. before the raw disks were added. After the disks were added to the solutions they were examined at frequent intervals in order to study the progress of maceration.

Experimental Data

The results of the various experiments with each organism at the different temperatures have been averaged and set forth in table 1. The length of time required to macerate the raw disks completely is given in hours. It is hardly necessary to point out that any method no more refined than the one employed here is certain to give some variation in the results. Some of the factors which have an influence on the results will be discussed later.

Table 1 shows that at only one temperature (20° C.) were data obtained on the maceration of the raw disks by all the organisms. At 40° and 30° the species *microsporus* and *nigricans* made no, or at least such a feeble, growth in the time allowed that neither the solution nor the mycelium contained a measurable quantity of pectinase. Likewise certain species, namely, *delemar*, *chinensis*, and *maydis*, made no growth at 9° in 26 days, the time allowed for development at the lower temperature. A small

amount of enzym was exuded into the substrate by *reflexus* at 40° and its action was determined, but the actual amount of mycelium produced was so small that its macerating power could not be measured.

TABLE 1. *Average rate of maceration by the enzym exuded into the solution upon which the different species of Rhizopus grew at various temperatures, as well as by that retained by the mycelium**

Temperature for Growth of Fungi (° C.)	*R. reflexus*	*R. delemar*	*R. oryzae*	*R. chinen-sis*	*R. nodosus*	*R. tritici*	*R. micro-sporus*	*R. maydis*	*R. nigri-cans*
In Solution									
40	10	4.7	4.7	4.9	3.25	4	.....	5.5	.....
30	2.75	2.1	2.15	4.5	2.05	1.45	.....	1.3	.....
20	2	2	1.5	5	1.75	1.25	1.75	1.25	4.87
9	2.5	.....	3.25	.....	1.87	2	24+	.....	48+
In Mycelium									
40	.....	8	8	8–24	4	4.5	.....	.....	.....
30	3	5	3.8	6	3.8	2.5	.....	2.5	.....
20	3	3	3.5	6.5	3	2.5	8	3	8–24
9	2.75	.....	2.5	.....	2.87	2.67	24	.....	24

* Figures indicate time in hours necessary to complete maceration.

The table shows that the enzym content of the solution was least when the different species were grown at 40° C., the time required to macerate the disks varying from 3.25 hours to 10 hours. *Reflexus*, a low-temperature form, made a very feeble growth at 40°. Under these conditions the total amount of pectinase produced by it was small in comparison with that secreted by the other species grown at the same temperature. On the other hand, the time required to macerate the disks was considerably shortened when the growth took place at 30°. This is particularly true of *reflexus*, which grew much better at the latter temperature. With respect to this temperature, it is interesting to note that *chinensis*, which, together with *microsporus*, was shown (1) not to be parasitic on sweet potatoes, required a much longer time to macerate the tissue than any of the other species. Both species, however, were shown elsewhere (2) to produce a pectinase when grown at 30° which would disintegrate raw sweet-potato tissue. At 20° all the species made a good growth and exuded a considerable quantity of enzym into the substrate. The time required for the enzym to complete maceration was less when the fungus was grown at this temperature than when it was grown at 30°, with one exception, *chinensis*, although the difference in time here is not great. Both *chinensis* and *nigricans* required two to four times longer than any of the other species completely to dissolve out the middle lamellae of the disks. At 9° several of the fungi made no growth. This is particularly true of the high-, and of some of the intermediate-temperature forms. The enzym exuded into the solution by *microsporus* and *nigricans*, which made a slight growth at 9°, required 24

and 48 hours, respectively, to complete maceration. A comparison of these two species when grown at 9° and 20° shows a decided decrease in the amount of pectinase produced at the lower temperature, although both are relatively low-temperature forms. The losses caused by the latter species frequently occur at a temperature not much higher than the lowest temperature used in these experiments. The tissues of potatoes decayed by *nigricans* under storage conditions are completely disintegrated, and the middle lamellae are dissolved out so that coherence is entirely lost, a fact which suggests that a pectinase is produced. Furthermore, the decay is rapid, and the dissolution of the middle lamellae takes place considerably in advance of the growth of the hyphae.

A study of the data derived from the use of the mycelium shows some interesting facts. It should be pointed out in this connection that the rapidities of maceration by the enzym in the solution and in the mycelium are not strictly comparable, since no attempt was made to use a quantity of enzym powder that would be equivalent in macerating power to the enzym contained in the solution. It will be seen that the mycelium of *oryzae*, *delemar*, and *chinensis* grown at 40° C. contained a small amount of enzym. *Microsporus*, one of the nonparasitic species, produced a small amount at 20° and at 9°. A comparison by this method of *chinensis* and *microsporus* with *nigricans*, a species which readily decays potatoes at 20° or lower, indicates that the latter produces a smaller amount of the macerating principle. The comparison of the results obtained with *microsporus* and *nigricans* in the solution at the same temperature shows similar results. There are some outstanding differences between the results obtained with the solution and those obtained with the mycelium. It has been pointed out that the amount of enzym in the solution increased with the decrease of temperature from 40° to 20°, and then decreased when the temperature was lowered to 9°. On the other hand, results obtained from the mycelium did not follow the same general course when the temperature was lowered from 20° to 9°. As a matter of fact, the results here show that, with one or two exceptions, there is more pectinase in the mycelium grown at 9° than in that grown at 20°. It is interesting to note in this connection that Harter (4) found amylase to be present in larger amount in the mycelium of *R. tritici* grown at 9° C. than at any higher temperature tried up to 40° C. On the whole, the results show that a larger amount of pectinase is produced per unit measure at intermediate to relatively low temperatures than at high ones. In two cases in which mycelium was used, *chinensis* at 40° and *nigricans* at 20°, the results are recorded in the table as varying from 8 to 24 hours. This merely means that some maceration had started in 8 hours, and that the process was completed in 24 hours, no further examination of the material having been made.

The results of these investigations show that, at least within the limits of these experiments, pectinase is produced at any temperature at which the organism grows. The amount produced is least at the higher and most

at the intermediate temperatures or in the vicinity of 20° C. Since this temperature is favorable for decay, it may be assumed that, within rather wide limits, the amount of pectinase produced is greatest at the temperature most suitable for the decay of sweet potatoes.

Maceration of Old and New Potatoes

Some of these investigations were conducted during the summer and autumn months, when either old sweet potatoes kept from the preceding crop or immature potatoes taken from the ground had to be used. The question naturally arose whether the old and the new potatoes would be macerated at the same rate.

It is believed by some investigators that sweet potatoes which have been in storage for some time are more readily decayed than those freshly dug, and there is some evidence which indicates that such is the case. The writers therefore decided to carry out a series of experiments to determine if there is any difference in the rate at which the middle lamellae of stored and freshly dug potatoes are dissolved. The experiments were conducted according to the method already described with all the species of Rhizopus, the organisms having been grown at 40°, 30°, and 9° C. and maceration carried out at 40°. At 40° and 30° the organisms were grown for 3 days, at 9° for 26 days. The old potatoes had been in storage for about 10 months. The new potatoes were freshly dug or had been in storage for only a few weeks at most.

The results of these investigations are shown in table 2.

Table 2. *Comparative rate of maceration of old and new potatoes**

Temperature for Growth of Fungi (° C.)	Kind of Potatoes	*R. reflexus*	*R. delemar*	*R. oryzae*	*R. chinensis*	*R. nodosus*	*R. tritici*	*R. microsporus*	*R. maydis*	*R. nigricans*
Solution										
40	Old	10	4.7	4.7	4.9	3.25	4	.....	5.5	.....
	New	12	5.25	5.5	12	3.75	4.5	.....	7	.....
30	Old	2.75	2.1	2.15	4.5	2.05	1.45	.....	1.3	.....
	New	4.5	4.25	4.5	6	4.25	2.75	.....	2	.....
9	Old	2.5	.....	3.25	.....	1.87	2	24+	.....	48+
	New	3	.....	5	.....	2.4	2.5	48+	.....	48+
Mycelium										
40	Old	.....	8	8	8–24	4	4.5	.....	.....	.....
	New	.....	7	7	14	3.5	4.5	.....	.....	.....
30	Old	3	5	3.8	6	3.8	2.5	.....	.....	.....
	New	3.25	5.75	5.25	14	5	3.25	.....	.....	.....
9	Old	2.75	.....	2.5	.....	2.87	2.67	24	.....	24
	New	4	.....	4	.....	4	4	48+	.....	48+

* Figures indicate time in hours necessary to complete maceration.

The results show that in nearly all cases the old potatoes are more readily macerated than the new. In some instances, for example, *chinensis*,

a nonparasitic species, the difference is quite marked. Unfortunately, some of the organisms would not grow at 40° and 30° C. and others at 9°, at least within the time allowed. It is evident from table 1 that *nigricans*, the principal cause of soft-rot of sweet potatoes, produces a very small amount of pectinase at 9°. The conclusion from the results shown in table 2 is that potatoes which have been cured and kept in storage for several months are more readily macerated than those freshly dug, the difference in most cases being very marked. The time required to macerate old potatoes is on an average about one half that required to dissolve the middle lamellae of new ones, as regards both the enzym exuded into the substrate and that retained by the mycelium. These results would seem to accord with the general observation that the susceptibility of sweet potatoes to decay increases with the increase in length of time they are held in storage.

Summary

1. The influence of temperature on pectinase production by the following species of Rhizopus was studied: *nigricans*, *reflexus*, *microsporus*, *delemar*, *oryzae*, *chinensis*, *nodosus*, *tritici*, and *maydis*. These experiments seem to indicate that the enzym is produced at any temperature at which the fungi will grow. Temperatures of 9°, 20°, 30°, and 40° C. were employed.

2. The amount of enzym produced was least at the highest temperature, as regards both that exuded into the substrate and that retained in the mycelium. The quantity of enzym in the mycelium was found to increase with a decrease in the temperature down to and including 9° C. Similar results were obtained with the solution, except that a slight reduction resulted in the quantity of enzym produced when the temperature was lowered from 20° to 9°.

3. The nonparasitic species (*microsporus* and *chinensis*) produced a considerable quantity of enzym, whereas *nigricans*, one of the parasitic species, produced a very small amount.

4. A comparison was made of the relative length of time required by the enzym produced by the different species to macerate the tissue of freshly dug sweet potatoes and of those which had been held in storage for several months. The fungi were grown at three different temperatures: 40°, 30°, and 9° C., maceration being carried out at 40°. In general, it was found that the middle lamellae of old potatoes were dissolved in about one half the time required to macerate the tissue of new ones.

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THE RELATION OF SOIL MOISTURE TO THE FUSARIUM WILT OF THE TOMATO[1]

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In an earlier article (3) the author has discussed the relation of temperature to the Fusarium wilt of tomato. In correlation with the temperature studies, inquiry was made into the relation of various amounts of soil moisture to the development of the disease. The earlier article includes an account of the disease and of the characters and source of the pathogen (*Fusarium lycopersici* Sacc.), as well as of certain details as to experimental methods. These statements will apply in general to the moisture studies also, since the work along the two lines was carried on simultaneously.

REVIEW OF LITERATURE

There are few records in the literature of plant pathology of controlled experiments dealing with the effect of soil moisture. However, a body of recorded observations offers suggestions as to moisture effects. In these it will be noted that the reference is sometimes directly to soil-moisture and at other times to soil aëration or drainage. Field observations have led to the belief that hot, dry weather favors the Fusarium wilt. Humbert (8) has recently made this statement, and, as he has supported the statement with meteorological data, there seems to be no reasonable doubt that practically every serious outbreak of the disease comes during hot, dry weather.

From a consideration of parasitic plant diseases in general, the most definite fact which has been brought out concerning soil-moisture effects has been that with saturation of the soil the host-parasite balance may be completely overturned. Recent literature indicates that usually this has resulted in a great increase in the amount of disease. Thus Johnson (9), in carefully controlled work with the Thielavia root rot of tobacco, found that saturation of the soil greatly increased the amount of disease. Rolfs (14) has reported that Rhizoctonia on a number of crops is most severe on poorly drained lands, and improvement of drainage has been recommended as a control measure for a wide variety of soil troubles. Hole (7), of the Indian Department of Agriculture, says that the sal-root fungus, *Polyporus shoraae*, is widely distributed throughout the sal forests of India, but that, so far as is known at present, it does damage only in those wet forests of Bengal and Assam in which conditions of soil aëration are known to be

[1] Investigations carried on at the University of Wisconsin under advisory relations with Professors L. R. Jones and E. J. Kraus, to both of whom the author is indebted for counsel and criticism.

particularly unfavorable. He further notes that by promoting a diseased and sickly condition in the roots, poor soil aëration may be a factor of great importance in facilitating the attacks of injurious root fungi of this class.

On the other hand, in a few instances diseases have been reported as being less severe with the soil saturated than otherwise. Appel (1) reports that alder trees in Germany suffer more severely from the parasite *Valsa oxystoma* when they are growing in meadow-land than when they are in their natural swampy habitat. Peltier (13) states that when carnation plants growing in soil inoculated with Rhizoctonia were given a heavy watering and the soil was then allowed to dry out, they were killed more rapidly than were plants growing under the same conditions, except that the soils were continually over-watered.

Concerning the effects of medium and low moistures there is very little which can be said definitely. Johnson found that whether the soil was one fourth, one half, or three fourths saturated made very little difference in the amount of Thielavia root rot which appeared.

The relation of soil moisture to the growth of autotrophic plants has been the object of considerable investigation, and in general the results indicate that there is a very wide range of moistures at which plants will grow well. Fowler and Lipman (5), reporting on work with lemon trees, state that the range of optimum or nearly optimum conditions of moisture is relatively wide. They also found that, as the moisture content was raised above the optimum, the growth curve broke sharply. As moisture decreased from the optimum, however, the rate of growth fell off very gradually. Thus the moisture curve was characterized by a gradual rise to the optimum and a sharp drop from the optimum to saturation. Kiesselbach (10) secured best growth of corn at 60-percent saturation. He noted also that the plants grew well from 20-percent to 98-percent saturation. Harris and Maughn (6), working with wheat, secured the highest yield of grain with a soil having a moisture content of 20 percent throughout the growing season, this being equal to approximately two-thirds saturation. The problem of soil moisture in relation to the growth and activity of soil fungi has received little attention. Waksman and Cook (15), and more especially Coleman (4), have published results of experiments in which they grew a variety of soil-inhabiting fungi in soil cultures of different moisture contents. The results showed that the moisture requirements of fungi differ considerably. Thus a dry medium favored maximum growth of *Aspergillus niger*, while *Trichoderma koningi* grew best on a moist medium.

Methods of Controlling Soil Moisture

Prior to beginning these experiments, a survey was made of the literature, with the object of securing information on the technique of controlling soil moisture. In what might be called the "practical" experiments, involving economic crops, the custom has been to weigh the plants, container, and

soil at frequent intervals, sufficient water being added at these times to replenish losses. With this method the moisture is not kept absolutely constant, the range of fluctuation depending on the frequency of weighing and the rate at which water is lost. In an effort to maintain soil moisture at constant, fixed values, and at the same time to do away with some of the laborious weighing, Livingston and his associates have devised the auto-irrigator. This makes good the loss of moisture from the soil as rapidly as this loss takes place. The auto-irrigator was given a trial, but it did not function properly with low soil moistures because of poor capillarity. The capacity of the auto-irrigator, furthermore, was insufficient to supply the needs of a large, rapidly transpiring plant.

After both these methods had been used with various modifications, the following combination was finally decided upon. With dry soils, *i.e.*, those having a moisture content of 20 percent or below, the ordinary paraffin-seal method was adopted, the water being introduced through a glass tube leading from the surface to an inverted pot buried in the soil. The rate of loss of water from these low-moisture crocks of soil was slow, and the soil-moisture content was kept constant by weighing every two or three days and restoring the original weight by the addition of water. Measured amounts of water were also added between weighings. The auto-irrigators, as described by Livingston and Hawkins (12), were installed in those crocks which were to be run with a medium (23 percent) to high (35 percent) moisture content. Soils held at these moistures did not have the surface sealed, the only covering being a layer of non-absorbent cotton. The crocks were weighed every other day, and water was added to the surface of the soil when necessary. The gross weight necessary for a certain percentage of soil moisture was known, also the weights of all materials other than soil. The method of moisture control, as outlined, was fairly satisfactory from the standpoint of manipulation and possessed certain other distinct advantages. The surface of the soil with a high moisture content was not sealed with paraffin, for in an early experiment sealing was found to exert an inhibitory effect on growth if the experiment was continued for two weeks or more. This was not found to be the case, however, with very low soil moisture.

Bergman (2) has recently shown that shortage of oxygen is a factor which limits growth in the case of saturated soils. Obviously, then, if one of the deleterious effects of high soil moisture is the limitation of oxygen supply to the roots, any technique which tends still further to limit the oxygen supply will affect the results of a moisture experiment. Thus, a soil having the pore spaces filled with water until aëration had been reduced to a minimum would respond in a very marked way if the surface was sealed tightly, such treatment under these circumstances having the same effect as more moisture. Under low soil moisture, oxygen supply is not the factor limiting growth, and even with the soil surface sealed there is abundant aëration.

The surface was sealed in this case in order to prevent the formation of a hard crust as the result of excessive evaporation from the surface and a break in the capillary movement from below.

Koehler (11), working earlier in this laboratory, found in quantitative experiments that growth with low soil moisture is not affected by sealing the soil surface, but that with higher soil moisture this sealing brings about a marked inhibition of growth. He also found that with very dry soil, in containers, there were great differences in moisture content at different soil levels, unless the surface was sealed. With the higher moistures, where capillary movement was very free, this did not hold except to a very slight extent.

In the manipulation of the soil to obtain uniformity in soil-moisture content, an important factor is the degree of compactness of the soil mass. Various writers have noted the possibility of error arising from differences in this respect. The method finally adopted in these experiments, in order to obtain relative constancy, was to pack the soil firmly into the crocks and set in the plants. These crocks were then allowed to stand for a month or more, by which time the plants were well established and the soil was very compact, as would be the case in the field a month or more after planting. Then, just prior to the beginning of the actual experiment, one or more of the crocks was saturated, water being added until a slight excess of free water remained on the surface. The value secured in this way was taken as the saturation point. This saturation point varied with the height of the containing crock and with the degree of compactness of the soil. In series II, low crocks freshly filled with soil gave a saturation value of 40 percent. In series III and IV, the same soil, in tall crocks which had been allowed to stand and settle for six weeks after they had been filled, had a saturation value of 35 percent. When the soil was actually saturated, whether this required 35 or 40 percent of moisture, the development of wilt disease was inhibited, while with the soil moisture just below saturation, whether the percentage content was 32 percent or 37 percent, the wilt developed rapidly. All percentages of moisture are expressed in terms of wet weight.

Experimental Results

Experiments I and II. These were in the nature of preliminary experiments and were not satisfactory in certain respects. In experiment I, conducted in the spring of 1919, the temperature was not sufficiently high to permit development of the wilt in a virulent form. The crocks were carried at low, medium, and high moisture, the actual percentages maintained being as follows:

Soil high in organic content:
18 percent, 27 percent, 40 percent (saturation).
Soil low in organic content (a sand loam):
11 percent, 20 percent, 30 percent (saturation).

Infection was secured at medium and low moistures, but none at saturation.

Experiment II was carried on the following fall; in this case the temperature conditions maintained were favorable for a maximum development of the disease. The regulation of moisture was not entirely satisfactory and the methods of planting and handling the crocks were not yet perfected. The results of this experiment, however, showed decisively that plants grown in saturated soils do not develop the wilt. With high moisture content which did not quite reach saturation (30–35 percent), the wilt developed, and the same was true with medium moisture content (25–30 percent). There was a distinct falling off in the amount of disease in soils which were dry enough (22 percent and below) markedly to check growth.

The results from this series are summarized in table 1.

TABLE 1

Soil Moisture, Percent	No. Plants	Percent Healthy	Percent Diseased	Percent Dead
18–25	19	47	31	22
26–27	27	15	59	26
40 (saturation)	12	100	00	00

Experiment III. This experiment was begun December 22, 1919, and completed March 1, 1920. The soil was steam-sterilized and inoculated with a spore suspension of *Fusarium lycopersici.* One-gallon crocks were filled, planted, and carried into a cool temperature (15°–20° C.) greenhouse. Here the plants grew for a period of four weeks, with an air temperature of 15° to 20° C. and a soil temperature averaging about one degree less. There was no development of the disease at these temperatures.

On January 19, 1920, a series of crocks containing inoculated plants was carried into a temperature favoring the disease (about 28° C.), the soil having previously been adjusted to various moisture contents.

On February 20 the condition of the plants was as shown in table 2.

TABLE 2

Moisture, Percent	No. Plants	No. Diseased	No. Dead
14–16	4	0	0
16–18	4	3	1
23–24	4	4	3
26–28	4	3	2
31–33	4	4	4
35 (saturation)	2	0	0

On March 1 the experiment was discontinued and the data taken are shown in table 3.

Experiment IV. The tomatoes used were planted December 22, 1919, and grown at the low temperature (15°–20° C.) until February 9, 1920.

At this latter date they were transferred to the warm-temperature house, the soil having first been brought to the moisture content desired for the experiment.

TABLE 3

Moisture, Percent	No. Plants	No. Diseased	No. Dead
14–16	4	1	0
16–18	4	4	1
23–24	4	4	3
26–28	4	4	3
31–33	4	4	4
35 (saturation)	2	0	0

On March 15 the experiment was concluded, and the data taken are shown in table 4.

TABLE 4

Moisture, Percent	No. Plants	No. Diseased	No. Dead
13–14	4	0	0
15–16	4	1	1
17–18	2	2	0
22–24	4	4	3
26–28	2	2	1
28–32	3	3	3
35 (saturation)	2	0	0

These experiments showed that the plants growing in the driest possible soil were highly resistant to the wilt disease in spite of the fact that these plants wilted badly during the middle of the day from lack of water. The most rapidly growing plants were attacked and killed first, while plants growing in saturated soil seemed immune since they were never attacked by the disease. During the progress of these experiments data were taken on the incubation period of the disease under the different conditions of soil moisture. The data given in table 5 are from experiment IV.

TABLE 5.

Moisture, Percent	No. Days Prior to First Appearance of Disease	Incubation Period (Average)
13–14	35	35
15–16	24	24
17–18	26	26
22–24	15	19
26–28	18	20.5
28–32	15	19
35 (saturation)	—	—

These data again bring out the point that the disease makes its most virulent development with medium and high soil moisture. The plants growing in dry soil were slow to produce visible signs of the disease, and the development of the trouble, even after it had affected the lower leaves, was very much retarded.

Experiment V. In the work up to this point the principal purpose was to determine the effect of constant differences in soil moisture, the moisture content ranging from very nearly the minimum for life to complete saturation. The problem of sudden shortage of moisture in connection with a rise in temperature which favored the disease was not directly attacked. However, as has been brought out, the plants growing in soil so dry that the foliage was in a semi-wilted condition most of the time were highly resistant to the wilt disease, while, on the other hand, the plants growing under the conditions of soil moisture most favorable to growth were the first to be attacked.

On January 15, 1920, fourteen one-gallon crocks were filled with sterilized soil which had been inoculated with a spore suspension of *Fusarium lycopersici.* The weights of the crocks, soil, clay cups, etc., were recorded, and two plants were set in each crock. These crocks were then kept in the cool greenhouse until March 13, at which time the first blossom clusters were well developed. At the end of this period the plants were moved into a compartment where the temperature (about 28° C.) was favorable to the disease, and there subjected to the following moisture conditions:

Two crocks were saturated first, the remaining twelve being divided into four lots of three each. The experiment was continued for a period of 5 weeks, as follows:

Lot A: The soil was kept moist for all 5 weeks.
Lot B: The soil was kept very dry for the first week and moist for the next 4 weeks.
Lot C: The soil was kept moist for the first week and dry for the 4 weeks following.
Lot D: The soil was kept very dry for all 5 weeks.

The plants of lot A made a very luxuriant vegetative growth; those of lot B were noticeably affected by the week of drought, but grew rapidly after this. Those of lot C grew very rapidly during the initial moist period, and suffered extremely when the soil was suddenly allowed to become dry. Many of the tender tips of the leaves dried up, and the stems became hollow. The plants of lot D were dwarfed and woody, and were practically the same size at the end of the experiment as they were at the beginning.

The data with regard to disease are summarized in table 6.

TABLE 6

Lot	Treatment		Length of Incubation Period in Days	No. Plants Dead or Diseased after 5 Weeks	No. Appearing Healthy
	Weeks Dry	Weeks Moist			
A....	0	5	15	6	0
B....	1	4	22	6	0
C....	4	1	24	1	5
D....	5	0	Experiment discontinued at the end of 35 days	0	6

The results of this experiment were in line with those secured previously, and, when considered in connection with the preceding work, make possible the following conclusions:

1. Plants growing very rapidly as a result of optimum moisture conditions for vegetative growth are most susceptible to the wilt.

2. Moisture shortage that checks the growth of the host plant checks the development of the wilt also, and the longer and more severe the period of drought, the more the disease is inhibited.

3. Plants growing in saturated soil are never attacked by the wilt disease.

The Relation of Soil Moisture to the Growth of Uninoculated Plants

Check series, consisting of plants grown in inoculated soil, were run with experiments I, II, and III. The data from check series III only will be considered here, as they are the fullest. This series consisted of single crocks, each containing two plants which were carried at the different soil moistures for a period of seven weeks with an air temperature of approximately 28° C.

The data given in tables 7 and 8 were taken at the conclusion of the experiment.

Table 7

Soil Moisture, Percent	Weight of Tops Produced		
	Wet Weight, Grams	Dry Weight, Grams	Percentage Dry Weight
14–16	14.7	2.4	16.3
16–18	23.5	3.1	13.1
19–21	75	9.6	12.8
23–25	102	11.37	11.1
31–33	146	15.6	10.6
35 (saturation)	15.7	2	12.1

Table 8

Soil Moisture, Percent	Microchemical Analysis of Stem								
	Tip of Plant			Base of Plant			Tap Root		
	Nitrate	Sugar	Starch	Nitrate	Sugar	Starch	Nitrate	Sugar	Starch
14–16	2	2	2	2.0	2	2	2	2	1
16–18	2	3	2	2.5	2	2	2	2	1
19–21	2.5	5	2	3	5	2	2.5	2	1
23–25	3	5	2	5	5	1.5	2.5	5	1
31–33	2	5	2	3	3	2	2.5	2	1
35 (saturation)	1	3	1.5	1	5	1.5	1.5	2	1

1, absent. 2, slight. 3, moderate. 4, abundant. 5, very abundant.

From these data it can be seen that the disease-resistant low-moisture

plants were high in dry weight and low in nitrates and sugars. The susceptible plants, grown under good moisture conditions, were rich in nitrates and carbohydrates but low in dry weight. The plants growing in saturated soil, which were immune to the disease, showed one striking difference from all others—they were almost destitute of nitrates.

It may be noted that this series was grown during the darkest portion of the year, January and February. In experiment III, which was conducted at the same time, the blossum buds were all abscised as soon as the plants were moved from the cool temperature (15°–20° C.) to the warm temperature (25°–30° C.). In experiment IV, carried on during February and March, there was abundant flowering at the medium and high soil moistures after the plants had been moved into the warm temperature. Since the moisture and temperature conditions were practically the same during the two experiments, this difference in flowering may probably be attributed to light conditions. In this locality (Madison, Wisconsin) the light is very much reduced for several months prior to the middle of February. After that time, however, there is a rapid increase in the amount of sunlight. This increase in sunlight facilitated carbohydrate manufacture and changed the carbohydrate-nitrogen ratio, with the result that the plants changed from the vegetative to the reproductive condition. This difference, however, was not correlated with a difference in the resistance or susceptibility of the plants to wilt, the behavior of the disease being the same in experiments III and IV.

Experiments Dealing with the Immunity Induced by Saturation of the Soil

After it had been definitely shown in experiment II that the plants growing in saturated soil were not subject to the wilt, the reason for this relative immunity was investigated. It was soon found that, if these plants in saturated soil were allowed to grow under conditions of medium soil moisture (25–30 percent) for a short time, their immunity to disease was lost. Thus, after the plants had resumed the "normal" type of growth, they were attacked by the disease and killed. It was further found that the period of time between removal of the plants from the saturated soil condition and the date of first appearance of the wilt was the same as the incubation period of freshly inoculated plants. This fact led to the conclusion that in the case of the plants in saturated soil the fungus was able to make little or no progress up the stem of the host as long as the soil remained saturated.

To corroborate these conclusions as to the inability of the parasite to grow in the tissues of the plants grown in saturated soil, these plants were taken out and dissected at different times. In several instances slightly discolored bundles were found in the base of the stem, but in no case was it possible to plate out the fungus from the stem. The lower roots of these

plants were found to be much decayed, only a few roots thrown out at the very surface of the soil being white and clean in appearance. Portions of partly decayed roots were plated out, and cultures of saprophytic fungi and bacteria were secured. In one instance, however, a root was plated out which gave pure cultures of *F. lycopersici*, the fungus apparently growing out from the decayed root cortex only. These cultures were preserved and proved through inoculation experiments to be virulently pathogenic.

These and other considerations have led to the view that the immunity induced by soil saturation is probably a host-plant factor. Thus the fungus grows very well in liquid culture media, and it may be present in the partially rotted roots. Furthermore, these plants throw out many new roots at the surface of the soil; through these roots infection could readily take place if infection were possible.

From microchemical analysis it was found that the saturation plants were markedly different from normal plants in their nitrogen relations. Resistance in these plants seemed to be correlated with the absence of nitrate nitrogen. This relationship was tested by growing plants in sand culture and adding nutrient solutions. A complete nutrient solution was supplied to part of the plants, and a solution lacking nitrate to the remainder. The temperature conditions in the greenhouse where this work was conducted did not permit a virulent development of the disease, but it was conclusively shown that plants grown without nitrate, the tissues of which plants give no nitrate test, are not infected by the fungus. Plants grown with a complete nutrient solution were readily infected.

Experiments Dealing with Resistance Induced by Low Soil Moisture

It has been shown by carefully controlled experiments that succulent, rapidly growing plants produced under optimum moisture conditions (23–33 percent) are subject to the disease, while the woody, slowly growing plants which result from low moisture (13–20 percent) are not readily attacked.

Rapidly growing, susceptible plants were made resistant by allowing the soil to dry, the growth being checked in this way. Slowly growing, resistant plants were made susceptible by making the soil moist, and thus inducing a rapid, succulent growth of the host. Thus, at the conclusion of experiment V there were set aside eleven plants which had been growing with the soil very dry and at a temperature of about 27° C. for five weeks without showing the wilt. The soil of four had been made moist; the remainder were allowed to continue dry. In a week the four plants in the moist soil had changed over into a succulent, rapidly growing condition, and in three days more all were showing the initial symptoms of wilt. The disease progressed rapidly, and soon the plants were completely wilted. The seven plants which had been allowed to remain dry during this time did not develop any disease and were in the same condition at the end of the experiment that they had been in at the beginning.

Thus the disease could be brought on by making the plants from the saturated soil (35 percent moisture) dry, or the plants from the dry soil (13–20 percent) moist. The reduction in amount of disease with dry soil and saturated soil appears in each case to be due to, or at least correlated with, detectable differences in the host plant. The resemblance between the two ceases, however, at this point.

When grown at a temperature favorable for the disease, and with dry soil, plants are infected and the fungus progresses slowly up the stem. If the temperature conditions are optimum for a sufficiently long period, these plants are killed by the wilt. In experiments IV and V the shortest incubation period for plants growing with 23 to 33 percent of soil moisture was, in each case, 15 days; the shortest incubation period for plants grown in the driest soil was 35 days. In numerous instances, apparently healthy plants growing with 13 to 14 percent soil moisture have been cut and found to have vascular discoloration extending far up the stem. The fungus was readily isolated from such plants, and in addition it was isolated in a number of instances from the tissues of plants which showed no browning of the bundles. These investigations have indicated that a very large majority of the low-moisture plants which do not show external symptoms of the wilt are nevertheless infected.

The presence of such infection can be readily shown in another manner. The temperature conditions were such that, with a soil moisture of 25 to 30 percent, the disease made its appearance in about 15 days. At the conclusion of experiment V, as already noted, four dry-soil plants which showed no disease were made moist; all four showed the disease in 10 days. Since it had been definitely proven that under the given conditions 15 days was the least possible time in which the fungus could infect, grow up the stem, and produce visible wilt, the only explanation for this result in 10 days is that infection had already occurred during the long period of weeks when the plants were growing in very dry soil, but at a temperature optimum for the disease.

Since the dry-soil plants are readily infected, and the fungus, once within the host, produces the disease very slowly, it seems reasonable to suppose that the host as a whole is resistant and that this fact is responsible for the great reduction in the amount of disease.

In the plants grown in saturated soil, infection did not occur, and the incubation period was not reduced by exposing the plants to a soil temperature between 25° and 30° C., for a long period with the soil saturated. There was no evidence that the disease would ever develop, or infection occur, so long as the saturated condition was maintained. For these reasons the dry-soil plants are characterized as resistant, while the saturated-soil plants are said to be immune.

Comparison of the Effects of Low Soil Moisture and Low Soil Temperature on the Host, in Relation to Development of the Disease

The resistance induced by low soil moisture seems different from that induced by low soil temperatures. Thus, plants could be produced which had tops that were susceptible and roots which were resistant to the disease, or roots that were readily invaded and tops that were not attacked. A soil temperature of 15° to 20° C. checked the progress of the fungus while it was in the roots, but exerted no influence if the parts above ground became infected, the temperature of the air being the deciding factor in that case. On the other hand, soil-moisture conditions low enough (14–18 percent) to check the disease exert an influence through the plant as a whole, roots and tops both being made resistant.

Temperature effects on disease were not correlated with apparent differences in the host plant; thus, the decrease from a minimum of disease at a temperature of 27° to 30° C. to no disease at 19° to 20° C. was not associated with a marked change in the appearance or in the composition of the host plants.

The case of soil moisture was different, for the decrease from a maximum of disease with a soil moisture of 30 to 33 percent to a minimum of disease with a soil moisture of 13 to 14 percent was correlated with a marked reduction in the vegetative vigor of the host plant. This decrease in the amount of disease did not begin to manifest itself until the soil moisture was reduced to a point where the plants actually began to show symptoms of moisture shortage, and the more acute this shortage became, the more pronounced was the decrease in disease. The plants grew well with soil moistures from 22 to 33 percent, and there was a virulent development of the disease. The plants growing with a soil moisture of 18 to 19 percent were the first to show perceptibly increased resistance to the disease, and likewise the first to show a marked reduction in vegetative vigor of growth. During periods of excessive transpiration they suffered severely. A soil moisture of 13 to 14 percent brought the plants close to the point of permanent wilting, and these plants showed a maximum resistance to disease. Thus the evidence now at hand indicates that soil moisture and soil temperature act in different ways to increase or diminish the amount of disease.

Interpretation of the Temperature and Moisture Results in Terms of Field Conditions

In the early work with temperature no attempt was made to control soil moisture accurately, water simply being added every day in amounts sufficient to keep the soil moist. In the later experiments the moisture was held constant by weighing the pots and adding the amount of water needed to restore them to the constant weight. However, since the range of moisture conditions almost equally favorable to the development of the disease

is very wide, 22 to 33 percent with the soil used in the moisture work, there was little object in controlling moisture exactly in the temperature experiments.

Early in the work, owing to the fact that the disease appeared to develop equally well over a wide range of soil moistures, it was thought that soil moisture was of very minor importance as compared with temperature. However, it now seems that extreme drought would certainly check the development of the disease, regardless of temperature conditions.

A brief consideration of the application of these findings to field conditions follows. The moisture results indicate that for a virulent development of the disease the plants must be rapidly growing. The temperature results showed that warm air and soil temperatures (27°–31° C.) were essential for a virulent development of the disease. Therefore it would be expected that, under field conditions, a rainy period, inducing rapid growth, followed by hot weather, would furnish optimum conditions for the quick appearance and the rapid progress of the disease. Weather which was hot and moist would be favorable, but it was pointed out in the temperature work that an even warm or hot temperature is not nearly so effective as the same temperature with intermittent periods of extreme heat. Bright, sunny days furnish this intermittent temperature, while during hot, moist weather the temperature is rather even. A warm, rainy period followed by hot weather would produce the disease more quickly than cool, rainy weather followed by a hot period, since under the former conditions infection would have occurred and growth of the fungus up the stem would have begun. Continuously dry weather which checks the growth of the plants should also check the development of the disease.

Summary

Tomato plants were grown in crocks of sterilized soil inoculated with a spore suspension of *Fusarium lycopersici*. The soil in these crocks was held at moisture contents ranging from 13 to 35 percent, the higher value representing complete saturation.

The plants growing in soil with a low moisture content, 13 to 19 percent, were very resistant to the disease.

The plants growing in soil which was kept saturated were immune from attack.

In general, it was found that any moisture shortage sufficiently severe to check the vegetative vigor of the host checked the disease proportionally.

When rapidly growing plants held at a temperature below 20° C. were brought into a temperature favoring the disease (25°–30° C.), they were soon attacked by the wilt. However, if the soil was allowed to dry out as soon as the plants were placed at the warm temperature, the appearance of the wilt was very much delayed. Thus, rapidly growing succulent plants, which had been susceptible to the disease, were made disease-resistant by allowing the soil to become very dry.

Plants growing in soil with a very low moisture content lost their disease resistance if a rapid, vegetative type of growth was induced by the addition of sufficient water to keep the soil moist.

Plants growing in saturated soil were immune to attack, but if the moisture content was lowered the disease soon developed.

The immunity of the saturated soil plants was apparently correlated with the practical absence of nitrates in the host tissues.

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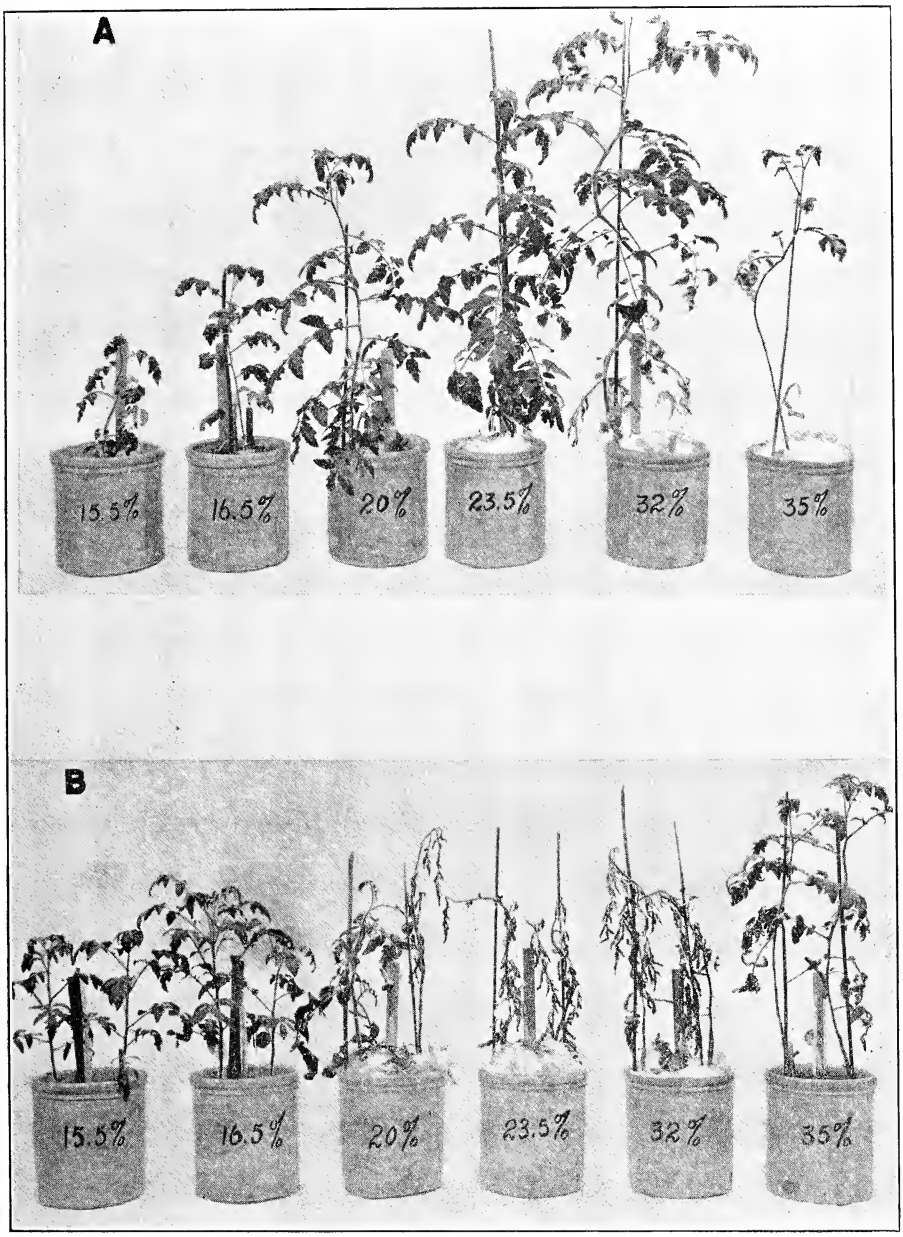
EXPLANATION OF PLATES

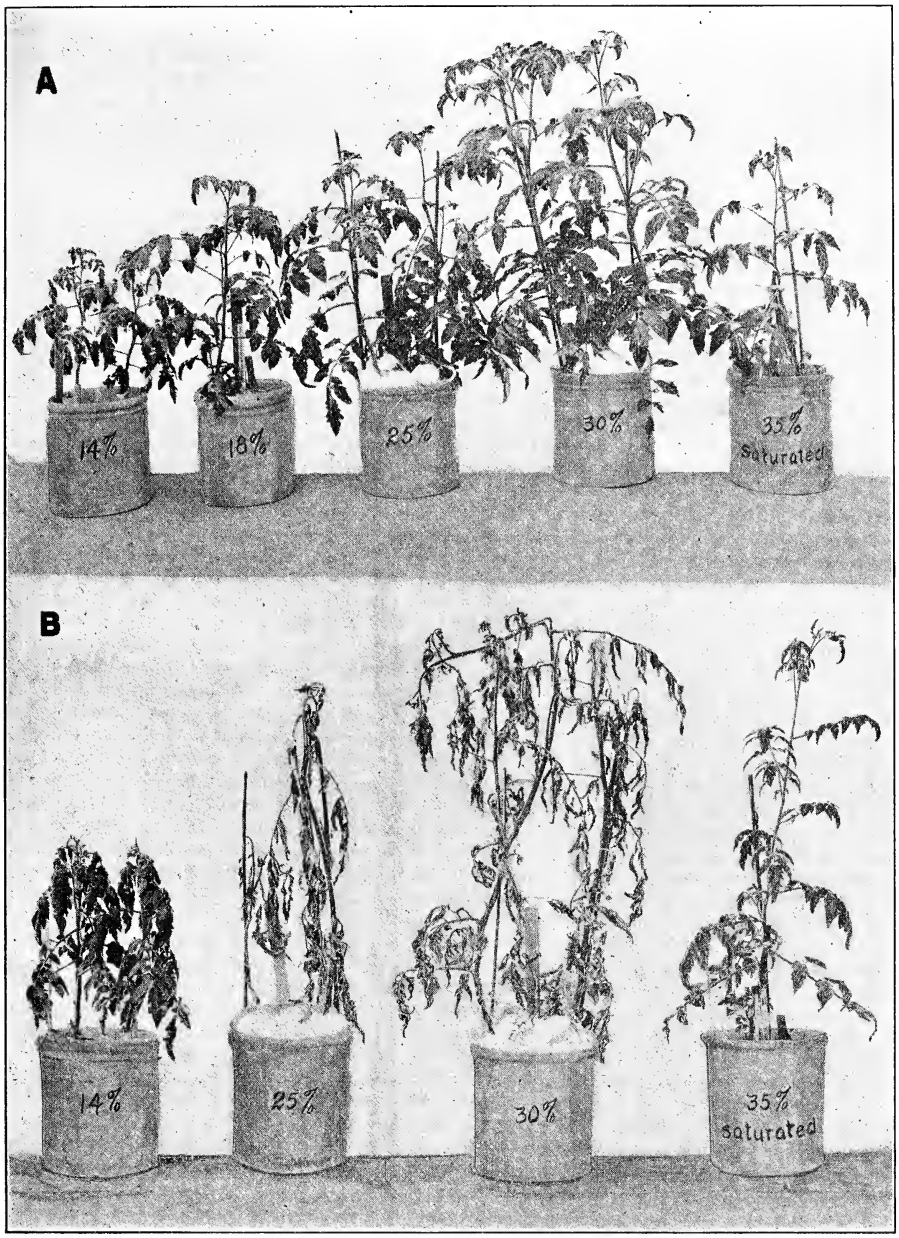
Plate XIII

A. A series of plants growing in sterilized, uninoculated soil with soil moisture ranging from 15.5 to 35 percent (saturation). At 32 percent, the maximum wet weight and the maximum dry weight of tissue were secured; the percentage dry weight, however, was the lowest at this percentage of moisture. The plants growing with 15.5 percent moisture were low in total weight but high in percentage dry weight. The plants growing in saturated soil (35 percent soil moisture) were low both in total weight and in percentage dry weight.

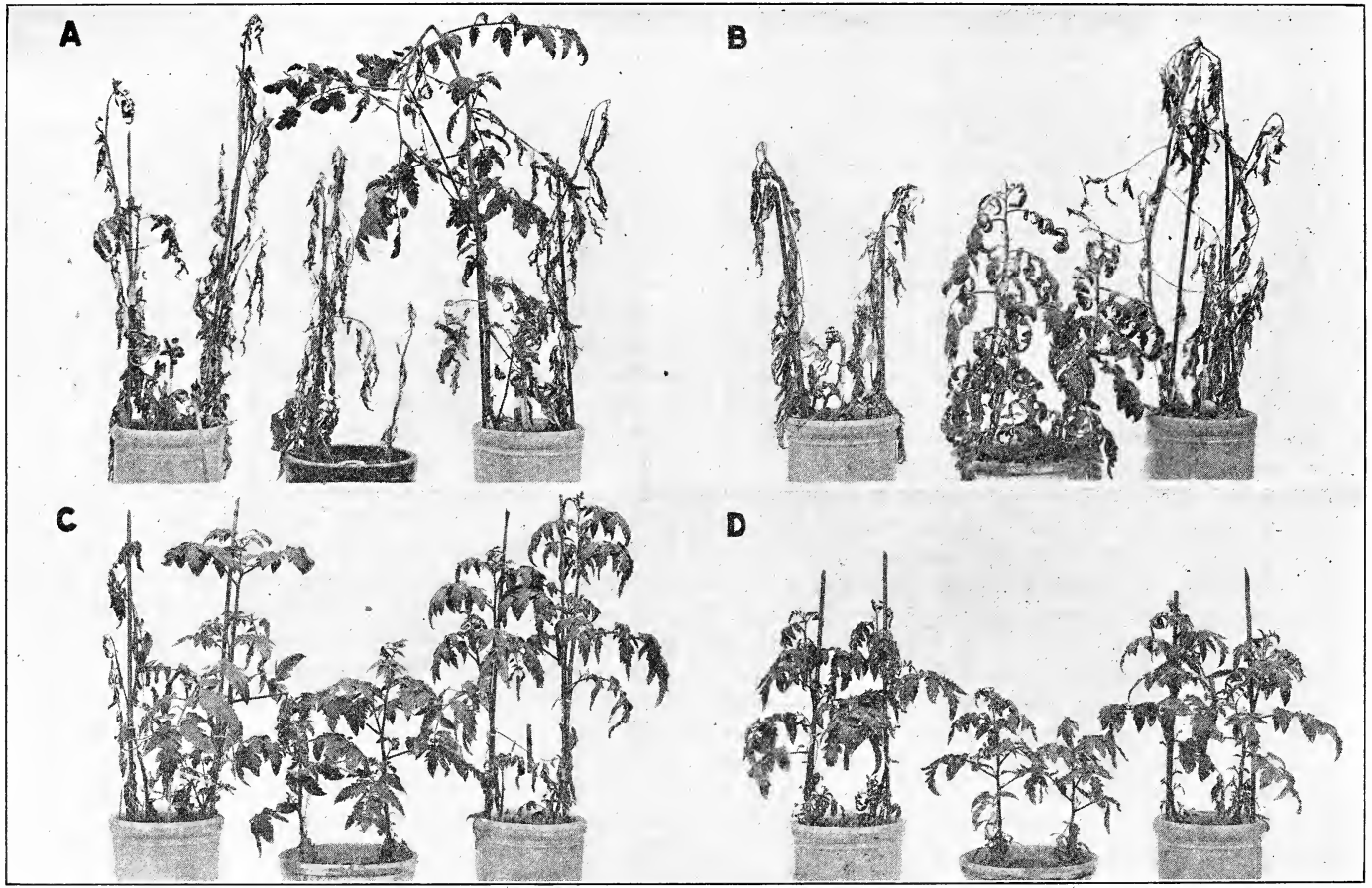
B. Plants grown in soils inoculated with *Fusarium lycopersici* at the same time as the plants of the check series (*A*), the soil moistures used in the two series being the same.



CLAYTON: SOIL MOISTURE AND FUSARIUM WILT



CLAYTON: SOIL MOISTURE AND FUSARIUM WILT



CLAYTON: SOIL MOISTURE AND FUSARIUM WILT

It will be observed that the disease affected the plants growing in soils with moistures from 20 to 32 percent. One of the plants in the 20-percent crock was still alive at the time the photograph was taken, while both plants in the 23.5- and 32-percent crocks had been dead and dry for some days.

PLATE XIV

A. These plants had been growing with moistures of 14, 18, 25, 30, and 35 percent (saturation) for two weeks. The soil was inoculated with *Fusarium lycopersici* in pure culture. Infection showed in the 25- and 30-percent crocks the day after the photograph was taken, and within two weeks these plants, which were making the most rapid growth, were dead. The temperature ranged from 25° to 30° C.

B. Four of the five crocks of plants shown in figure *A*, the 18-percent soil-moisture crock being omitted because of lack of space. This photograph, taken two weeks after the one above, shows how the wilt disease attacks and destroys tomato plants growing under good conditions, while plants growing in very dry soil (14 percent) or saturated (35 percent) were not attacked. The semi-wilted condition of the plants growing in the crock of soil held at 14 percent soil moisture is due to moisture shortage, not to attack by the disease.

PLATE XV

The plants in lots *A*, *B*, *C*, and *D* were grown five weeks in sterilized and inoculated soil, with temperature conditions favorable for the development of the disease.

The following sets of soil-moisture conditions were maintained:

Lot *A*: 5 weeks with moist soil.
Lot *B*: 1 week with dry soil, 4 weeks with moist soil.
Lot *C*: 4 weeks with dry soil, 1 week with moist soil.
Lot *D*: 5 weeks with dry soil.

The photographs were taken at the end of the five-week period. The disease developed with maximum virulence in lot *A*; in lot *B*, the plants were almost as severely affected; lot *C* had only one plant affected by the wilt; lot *D* showed no disease. The development of the disease was thus directly in proportion to the amount of moisture supplied. It is interesting to note that in lot *B*, one week of dry soil conditions having preceded four weeks of moist soil conditions, the incubation period for the disease was 22 days, while in lot *A*, good moisture conditions having been maintained for five weeks, the incubation period was 15 days.

STUDIES IN THE MORPHOLOGY OF *RICCARDIA PINGUIS*

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Riccardia pinguis (L.) S. F. Gray is found rather sparingly along the edge of the marsh next the wooded upland south of Lake Wingra (Madison, Wisconsin). The thalli grow on fallen willow canes, on the bases of standing willows, on the black mud, and occasionally on fallen leaves. In this locality they are well shaded during the summer when the willows and oaks are in full foliage, but are partly exposed during the fall and early spring.

Plants of this species are somewhat more abundant on the railroad right-of-way through the swamp prairie bordering Lake Waubesa and extending westward for about a mile. At this station the thalli grow on the mud and cinders and more rarely on the roots and stubble of grasses. The grasses stand one half to one and one half meters high and form a fairly thick shade over the thalli during the summer months, but are burned off annually in the fall or spring. Numerous searches in the swamp on either side of the railroad have revealed no plants beyond the limits of the annual burning of the grass.

In June, 1921, before the latter station had been discovered, the low prairie in the vicinity of Roby, Indiana, about twenty miles from Chicago, was visited in the hope of obtaining material for cytological study. Plants were found in this region growing chiefly on decaying leaves about the bases of very small willows scattered through the more swampy portions of the prairie. This material was not in thriving condition because of insufficient moisture, and only a small collection was made.

At all these stations the plants are protected from the summer sun but are favored with nearly full sunlight during the fall and early spring—the seasons during which vegetative growth appears to be most rapid. It was further observed that plants exposed, by tramping of the grass, to the August sun quickly succumbed.

Field and Culture Observations

The first collections of plants for this study were made in the spring of 1920 from the region of Lake Wingra and consisted of only a few dozen thalli. These plants were used to start greenhouse cultures which grew very well during the summer and early fall of 1920, but later became contaminated with blue-green algae and had to be discarded early in the following winter. These cultures were grown on a thin layer of leaf mold over a substratum of sand in shallow wooden boxes standing in a tray of nutrient

solution (formula of Moore, 1903). One of these cultures, started with about a dozen small thalli identified as female, spread over and completely covered an area of about one and one half square decimeters. It produced hundreds of archegonia but bore no sporophytes, there being no male plants in the culture. Another culture, male and female mixed, in a box four decimeters by three decimeters in area, completely covered the ground, mostly with several layers, and spread over the edges of the box. This culture was cared for by Dr. W. N. Steil during my absence of twelve weeks in the summer of 1920, and upon my return in September it was noticed that the male plants were being covered by the overgrowing female plants, in which young sporophytes were appearing rapidly. A few weeks later the culture appeared to be all female with developing sporophytes in large numbers. Most of the sporogonia when nearing maturity were eaten by insects, and the thalli succumbed to algal overgrowth.

Living plants bearing mature sporophytes were received February 11, 1921, from Mr. Severin Rapp, of Sanford, Fla. These, with the decayed wood on which they had grown, were placed on leaf mold underlain by sand in shallow earthen pots and plates and were saturated thoroughly with nutrient solution. On the day following they appeared to have recovered from the effects of shipping, and about two hundred of the youngest sporogonia were fixed in Flemming's solutions: the medium, the strong, and the strong diluted with an equal volume of distilled water. On sectioning these later, a few were found to show stages of the heterotypic and homoeotypic divisions; in others these divisions had been completed, and in some they had not yet begun.

A few of the sporogonia had already opened when they were received, and most of the remaining three or four hundred (estimated) discharged their spores within three weeks. Miss Clapp (1912) states that the spores when discharged often adhere in tetrads, but I have been unable to confirm this observation. The discharge of about a dozen capsules was observed under the binocular dissecting microscope; the spores appeared perfectly spherical except for the echinate surface and none could be found adhering in tetrads. However, it has more recently been found possible to obtain viable spores in tetrads by soaking the ripe capsules for several hours in water and then opening them under water.

Culture experiments with this plant have generally been unsatisfactory, and I have not yet succeeded in growing mature thalli from spores.

Two of these cultures of Florida material are still in thriving condition after sixteen months, although the plants have not grown very extensively. One culture, in a pot nine centimeters deep by eleven centimeters in diameter, appears to be almost entirely female, but has produced relatively few sex organs. The other culture, in an earthen saucer five centimeters deep by twenty-eight centimeters in diameter, is mixed, some parts being predominantly male and other parts mostly female. The plants in this

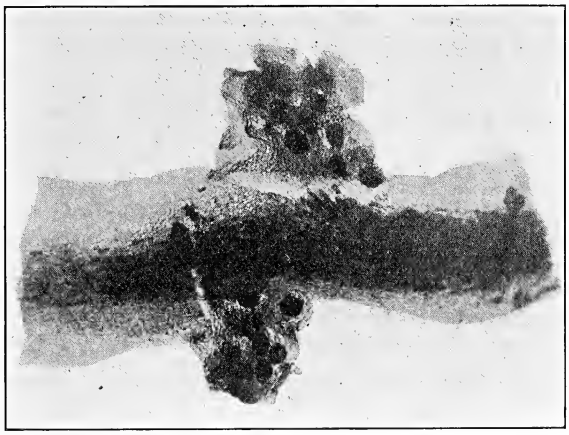
culture began producing sex organs early in the spring (1921) and produced great numbers of antheridia and archegonia until December, except during the hot summer months when the plants grew but little and appeared about to succumb. Numerous attempts to obtain functional antherozoids resulted only in the discharge of imperfectly formed, non-motile, coiled bodies. A few female plants with archegonia were fixed late in December and showed disintegration of the axial row before the maturing of the archegonium. No sporophytes have appeared in these cultures.

The form of the antheridial branches in these cultures was noticeably different from that of the antheridial branches of plants collected in the vicinity of Madison. These branches in the Florida material grown in the greenhouse were borne in groups of three (rarely five) as figured by MacVicar (1912, p. 50). The individual branches were short and wide, with thin upturned or inrolled margins. The antheridia were arranged rather irregularly. Representative plants were sent early in the spring (1921) to Professor A. W. Evans. He wrote that "the male branches are certainly very peculiar and I have never seen anything quite so indefinite among the various specimens of Riccardia which I have studied." He also commented upon the wide distribution of this species and suggested that "under the circumstances a considerable range of variation is to be expected."

The antheridial branches produced in these cultures from early spring to December showed no significant variations in form; but a few male plants sent by Mr. Rapp from the same locality in Florida and received October 8, 1921, bore antheridial branches so different from those of the Florida plants grown in the greenhouse that they might easily have been taken to be of a different species. The plants in this latter shipment bore antheridial branches ranging up to four or five millimeters in length, very uniform in width from base to tip, and with two (occasionally four) alternating rows of pits from which the antheridia had disappeared and which were arranged with almost mathematical regularity. Miss Clapp (1912), working on material collected at Xalapa, Mexico, described the arrangement of the antheridia on the branches as "extremely regular, in two alternating rows corresponding to the segments of the apical cell." The appearance of the plants in this latter shipment from Florida is certainly in accord with her description. In those branches having four rows of pits it seems evident that each branch had possessed two apical cells, the segments from each apical cell having given rise to two rows of antheridia. In the plants collected in the vicinity of Madison, the antheridial branches are generally borne singly, are relatively short and wide, and show little regularity in the arrangement of the antheridia (text fig. 1). It seems probable that the form of the sexual branches as well as that of the vegetative shoots in this species is readily affected by environmental conditions.

The most fruitful source of material (that near Lake Waubesa) was discovered August 11, 1921, when the plants bore mature sex organs in

great numbers and young sporophytes were fairly numerous. On this and the following day ten lots of material were fixed in the field, using Flemming's fixing solutions: the medium unmodified, and the strong diluted with an equal volume of distilled water. These gave equally good results; there was a slight shrinkage of the cells of the surface layer of the thallus and of the



TEXT FIG. 1. Photomicrograph of a portion of a male thallus bearing two antheridial branches, unstained, mounted *in toto* in balsam. × 12½.

cells of the archegonia, and in most cases considerable shrinkage of the cells of the young embryos. Thalli on which sporophytes were observed to be developing were generally left undisturbed in the hope that material might later be available for a study of the reduction divisions. After washing and dehydrating to eighty percent alcohol, the plants were picked over carefully under the binocular microscope and the male and female plants were separated.

The male plants in these collections averaged about one half to two thirds the size of the female plants (this is true also of a small collection of male and female plants, which had grown intermingled, collected one week earlier in the region of Lake Wingra). Two weeks later, when the Waubesa region was again visited, very few antheridia were to be seen, and the male plants were growing vigorously. Subsequent observations, when the male and female plants could be distinguished only by the vestiges of sexual branches or by the presence of sporophytes, have convinced me that there is no pronounced difference between male and female plants as to size or luxuriance of growth except during the period of gamete production, and I am not sure that the difference during this period is always so distinct as it appeared to be in the collections just described. Attempts to test this point by extended cultural experiments have thus far failed.

The plants fixed in the field August 11 and 12, 1921, near Lake Waubesa form the basis of the studies reported in this paper. This material was supplemented by greenhouse plants of both Wisconsin and Florida stock, but, except as otherwise stated, all figures were drawn from the material of these collections. The plants were imbedded in paraffin, cut into sections 10 μ thick, and stained either with Flemming's triple stain, Heidenhain's iron-alum haematoxylin, or with safranin and light green. For the observations recorded in this paper, the last-named combination proved most satisfactory.

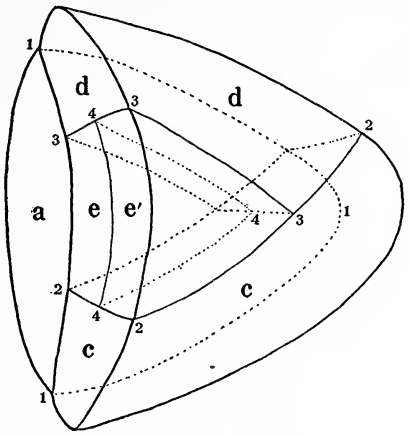
The Apical Cell and Segmentation

Miss Clapp's (1912) description of the position, form, and segmentation of the apical cell is in harmony with the earlier accounts of Kny (1863) and Leitgeb (1877). The apical cell is a large, wedge-shaped structure with right and left cutting faces from which segments are cut off alternately (Clapp, 1912). These two cutting faces are convex and meet above, below and behind, thus constituting all the surface in contact with other cells of the thallus. The free (anterior) surface of the apical cell is relatively small and approximates an ellipse of which the longer axis is the one perpendicular to the surface of the soil or other substratum on which the thallus is growing (fig. 4, Pl. XVI). Transverse sections through the apical cell show this same elliptical outline. Figures 4–8 show serial sections of the same apical cell (*a*) sectioned transversely, each section being 10 μ in thickness. This cell tapers abruptly to a blunt point in the next serial section, not shown in the figures. (Compare also the horizontal section shown in figure 1, Plate XVI, and Miss Clapp's figures 5, 6, and 11.) The relative lengths of the different axes of the apical cell vary considerably, but the horizontal transverse (right-left) axis is always much the shortest of the three principal axes (figs. 1, 4–8; Miss Clapp's figs. 5, 6, 11). In the vegetative branch the vertical and horizontal axes of the apical cell are approximately equal (figs. 3, 10). The apical cell *a* shown in figures 4–8 appears in six vertical transverse sections, each 10.μ in thickness, thus having an anteroposterior length of about fifty microns, approximately equal to the length of its median vertical axis (fig. 6).

The division of the apical cell is vertical and almost longitudinal (figs. 1, 11), cutting off a segment which at first approximates the shape of the apical cell (figs. 1, 9–11). Such segments are formed alternately from the right and left cutting faces of the apical cell, as all previous workers agree (see especially Kny, 1863, Pl. VI, figs. 4, 5; Leitgeb, 1877, Pl. I, fig. 1). The segment appears to exceed the apical cell in size, and the side of the segment adjacent to the apical cell soon becomes markedly concave, as the apical cell resumes its biconvex shape.

Leitgeb (1877), Campbell (1905), and Miss Clapp (1912) differ in their accounts of the division of the primary segment. Leitgeb (p. 41) says that the primary segment divides like a two-faced apical cell, cutting off segments

not to the right and left, but below and above. These divisions are best represented by a diagram showing the apical cell, the primary segment, and the cells into which the primary segment is divided (text fig. 2). In this diagram the plane 1–1 · · 1 represents the division which cuts off the



TEXT FIG. 2. Diagram of the apical cell (*a*), and of the cells into which the primary segment divides; explanation in text.

primary segment from the apical cell *a*. The first division (2–2 · · 2) of the primary segment cuts off a ventral cell *c* (see also figs. 9, 13, 2, 4, Pl. XVI). The next division (3–3 · · 3) cuts off a dorsal cell *d*, and intersects the preceding division wall (see also figs. 2, 4, Pl. XVI). The third division (4–4 · · 4) is vertical, at right angles to the former two, and divides the middle cell into right and left halves, *e* and *e′* (cf. fig. 4, Pl. XVI). Apparently one of these last two cells may continue to divide as an apical cell, giving off right and left segments and becoming the axis of a new branch; or both cells *e* and *e′* may function as ordinary surface cells. I have not followed these later divisions critically, but all my observations are in perfect agreement with those of Leitgeb. Figure 9 shows a vertical longitudinal section of the primary segment (*b*) in process of its first division. This figure was drawn from the serial section adjacent to and preceding the one shown in figure 10. Figure 13 shows the secondary segments (*b′* and *c*) in an archegonial branch, and was drawn from the serial section adjacent to and following the section shown in figure 12. Figure 2, drawn from the section adjacent to and following the one shown in figure 3, shows the secondary segments (*b′* and *c*) in preparation for the next succeeding division.

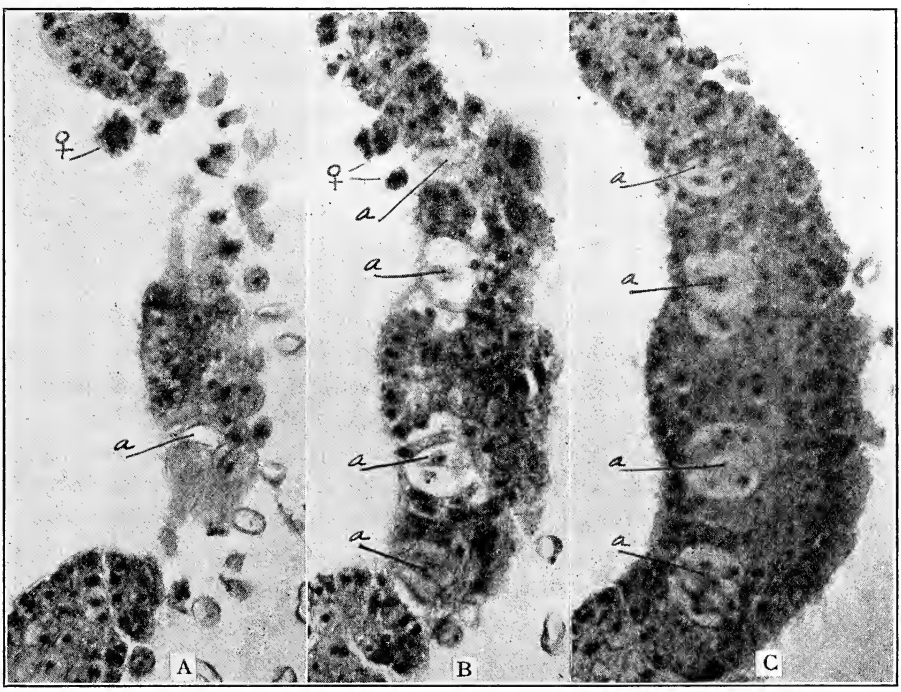
Miss Clapp reports that "the primary segment is divided by a vertical transverse wall into an inner posterior cell and an outer anterior marginal one." I have observed this first division of the primary segment in a half dozen or more clear cases. All of these show this division wall in a diagonal plane intermediate between the horizontal plane and the vertical transverse

plane of the thallus, intersecting the outer exposed wall of the cell and sloping upward interiorly (figs. 2, 9, 13). In some of the cases observed the plane of division is more nearly horizontal than indicated in figures 2 and 9. It is clear that, if seen in horizontal sections, the secondary segments shown in figure 2 and those in process of formation in figure 9 would appear to be "inner posterior" and "outer anterior" cells; this is the case in the horizontal section shown in figure 1, in which *c* and *b'* are the daughter cells in question. Miss Clapp figures only the horizontal sections (see her figures 3–10), and I am unable to determine with certainty from these whether in her material the division of the primary segment is a vertical transverse one, or whether, as I have found in harmony with Leitgeb, it is really in a diagonal plane.

Campbell says that "the segment first divides into an inner and an outer cell, and the former probably next into a dorsal and a ventral one." He does not figure these divisions, and it is not clear to me whether he means that the first division of the segment is into an inner posterior and an outer anterior cell, or into an inner cell proximal to the apical cell, and an outer cell distal to it. His description of the origin of the sex organs seems to imply that he means the latter, but it is difficult to reconcile his statement with the observations of Leitgeb, or with those recorded in the present paper.

As described by Miss Clapp, the apical cell is located in a sinus resulting from the forward growth of the thallus to the right and left of this cell. Each sinus contains usually two or more apical cells, which gradually diverge as new segments are cut off and undergo further division. As the two apical cells thus diverge, the group of cells between them grows forward and divides the sinus into two; but in the meantime each apical cell usually has given rise to another, so that each new sinus contains two apical cells. The time of the formation of new apical cells seems to vary, however, so that a sinus may contain more than two or (rarely) only one apical cell. The branching is truly dichotomous, but usually one branch of each pair develops only slightly and thus becomes apparently lateral (in this connection see also Clapp, 1912, and Campbell, 1905). During the season of gamete production these lateral branches begin very early to produce sexual organs, thus becoming gametophores. Text figure 3 shows a series of transverse sections, 10 μ in thickness, of the growing end of a thallus having four apical cells (*a*) in one sinus. The portion of the thallus in the middle of the sinus had just begun to push forward in advance of the apical cells, and two young archegonia (♀) shown in photographs *A* and *B* indicate that the two apical cells uppermost in the photographs were initiating an archegonial branch. The apical cell and the young cells immediately adjacent to it are much larger than the older cells a little farther removed from the apical cell and contain relatively few plastids. In the haematoxylin preparations the cytoplasmic portions of these cells are stained only lightly (text fig. 3).

The more rapid growth of the dorsal portion of the apical region tends to push the apical cell toward the ventral side (fig. 3, Pl. XVI). This tendency is especially noticeable in the greenhouse plants grown from the Florida stock (fig. 10; see also Miss Clapp's fig. 2).



TEXT FIG. 3. Photomicrographs of successive transverse sections of the apical region of the thallus; sections 10 μ in thickness, stained with haematoxylin. × 175.

DEVELOPMENT OF THE ARCHEGONIUM

The apical cell of an archegonial branch gives rise to relatively few segments, and these branches are consequently so short that it is rarely possible, in examining material *in toto*, to detect any regularity in the arrangement of the archegonia with reference to the axis of the branch. In sections of these branches there appears some evidence to confirm the statements of Miss Clapp for this species, and of Campbell (1905) for the Anacrogynae in general (not including Sphaerocarpales), that each primary segment of the apical cell gives rise to an archegonium. Figures 12 and 13 represent successive vertical longitudinal sections through the short archegonial branch and show the apical cell (*a*), a three-celled archegonium, an archegonium with egg and ventral canal cell formed, and a tangential section of a mature archegonium.

Several thousand archegonia in various stages of development were sectioned and stained in an attempt to obtain the history of the behavior of

the gamete nuclei in fertilization. These preparations afford a favorable opportunity to trace in detail the development of the axial row which seems to be slightly different from that of any of the Hepaticae as yet described in complete detail.

The stages preceding the division of the primary axial cell to form the cover cell and the mother cell of the axial row (terminology of Durand, 1908) have not been examined exhaustively, since these stages are not numerous in my preparations and since the cells in these stages are frequently shrunken somewhat by the action of the fixing reagents. Those cases observed seem to be in harmony with the general account of this development in the Hepaticae and with Miss Clapp's account for this species. I have not attempted to trace the subsequent history of the cap cell, but such observations as I have made seem to indicate that its behavior is so nearly like that of the other wall cells as to make it difficult to distinguish from them.

The mother cell of the axial row grows somewhat before its first division into the central cell and the neck-canal mother cell (figs. 19–22, Pl. XVII). These two cells grow to be relatively large before the next division, which occurs in the neck-canal mother cell (fig. 23) and leads to the formation of an axial row of three penultimate cells (fig. 24). Soon afterward the central cell divides, apparently equally, forming the egg and ventral canal cell (figs. 25–27). This division is followed by a period of growth, especially in the egg (fig. 28). Archegonia at this stage of development are fairly frequent in my preparations. Later the penultimate cell next above the ventral canal cell divides (figs. 29, 30, 38, Pl. XVIII); this division being followed shortly by a division of the uppermost of the penultimate cells of the axial row (figs. 30, 31). The final result is an axial row of six cells—egg, ventral canal cell, and four neck-canal cells. The last two divisions are not frequent in my preparations, and I have not been able to determine whether cell division follows nuclear division in all cases as it seems to in the penultimate cell next above the ventral canal cell shown in figures 29, 30, and 38, followed very soon by a disappearance of the division membrane, or whether the division of the cell may be omitted, as is suggested by such conditions as are represented in figure 31, Plate XVIII, and figures 42 and 43, Plate XIX. Durand (1908) finds that in Marchantia the nuclei of the neck-canal cells sometimes divide just before the disintegration of these cells, these nuclear divisions not being followed by cell divisions. The scarcity of archegonia showing the ultimate cells of the neck-canal row is probably accounted for by the short duration of these cells (Clapp, 1912; Florin, 1918).

I have been unable to detect a cell wall between any two of the cells of the axial row, except usually a thin film between the egg and the ventral canal cell. This film seems to be continuous with the walls of the cells of the venter (fig. 27, Pl. XVII; figs. 32, 34, 35, Pl. XVIII) and stains (with light green) like a cell wall. In disintegrating, the protoplasts of the canal cells probably form a hydrophilous colloidal mass which by its swelling forces open the end of the neck (fig. 33, Pl. XVIII; figs. 39, 40, Pl. XIX).

The cells of the axial row show an alveolar cytoplasmic structure, the alveoli becoming larger as the cells grow older. My preparations in general do not show the plastids clearly, and only rarely can these structures be seen distinctly in any of the cells of the axial row. They are, however, visible in some preparations, both in the cells of the axial row and in fairly young embryos. A few nearly mature eggs show bodies which are probably plastids, and if, as Miss Clapp reports, starch grains are present in the mature egg, there seems little reason to doubt that plastids are present in the egg and zygote throughout their history.

Occasionally, instead of three penultimate cells (central cell and two neck-canal cells) in the axial row at the stage shown in figure 24, Plate XVII, as described above, four are formed. All of these four divide in acropetal succession as do the three of the more frequent type, and this division results in the formation of an egg, a ventral canal cell and six neck-canal cells (figs. 34–37, Pl. XVIII). (See also Clapp, 1912, and Florin, 1918.) In the two archegonia represented in figures 36 and 37, there is no indication of a cell division following the last nuclear division in the neck-canal cells, but the egg and ventral canal cell have evidently begun to disintegrate and these cases cannot be regarded as normal. Axial rows of four penultimate cells are not sufficiently frequent to justify an unqualified statement as to the manner of the formation of the fourth penultimate cell, but the evidence available seems to indicate that the uppermost cell of the three-celled axial row grows and divides before, or at about the same time that, the central cell divides into the egg and ventral canal cell (figs. 34, 35). Another variation of similar type but of inverse order is represented by a single case (fig. 32) in which the usual first division of the neck-canal mother cell was omitted, thus giving rise to only two penultimate cells, one of which has divided to form a typical egg and ventral canal cell and the other is in preparation for division to form a neck-canal row of only two cells.

The exact sequence of divisions in the development of the axial row has not been followed in a large number of the Hepaticae, but certain incomplete accounts seem to indicate that in some of the other Anacrogynae that development is similar to this in Riccardia. Janczewski (1872) described the central cell of *Pellia epiphylla* as remaining undivided while its sister divides to form eight (rarely nine) neck-canal cells. The central cell then divides to form the egg and ventral canal cell, this division being followed by a doubling of the number of cells in the neck and in the neck-canal row, making sixteen (occasionally eighteen) of the latter. He does not say whether the division of the central cell is equal or unequal, nor does he explain the manner of the doubling of the number of cells in the neck-canal row. If, as seems probable, this doubling is brought about by a division of each of the eight (or nine) cells, the egg and ventral canal cell, as in Riccardia, are the oldest of the ultimate cells of the canal row. Hutchinson (1915) reports that the maximum number of neck-canal cells he could find in *Pellia epiphylla*

was nine. It is conceivable that the last division of the neck-canal cells was not shown by his material.

Janczewski reported also that archegonial development in *Fossombronia pusilla* differs from that in Pellia only in the smaller number of neck-canal cells, the central cell dividing when there are four neck-canal cells which later increase to eight (probably by a division of each of the four). This author reported also that "*Jungermannia* [*Lophozia*] *excisa* and *Radula complanata* agree exactly with Fossombronia."

However, he found conditions somewhat different in the Marchantiales. In *Riccia Bischoffii* the axial cell, after the cap cell has been cut off, divides to form the central cell and a neck-canal mother cell. The latter by two successive divisions gives rise to four neck-canal cells, and the former divides, just before fertilization, into a large egg and a small ventral canal cell. In *Preissia commutata*, *Marchantia polymorpha*, *Reboulia hemisphaerica*, *Lunularia vulgaris*, and *Plagiochasma Rousselianum* the process is reported to be similar except that in Marchantia the division of the central cell occurs somewhat earlier, sometimes even before the division of the penultimate cells of the neck-canal row.

Garber (1904) has followed in detail the development of the archegonium of *Riccia natans* and confirms the observations of Janczewski, adding that the central cell grows rapidly during the period before its division.

The detailed account of archegonial development in *Marchantia polymorpha* given by Durand (1908) confirms all points reported by Janczewski and adds that the ultimate neck-canal cells sometimes show a belated nuclear division which is not followed by a division of the cytoplast. Haupt (1921) confirms Janczewski as to the manner of the division of the central cell in Reboulia and as to the number of neck-canal cells present at the time of this division. He finds, however, that these cells (four in number) are later increased to eighteen or twenty.

The more modern studies on members of the Jungermanniales reveal probably a greater variety of conditions than Janczewski suspected, though none of them give the details of the development of the axial row. Humphrey's (1906) work on *Fossombronia longiseta*, although only fragmentary as concerns the archegonium, seems not to accord with the former author's observations on *Fossombronia pusilla*, or with the conditions found in *Riccardia pinguis*. The work of Haupt (1920) on *Fossombronia cristula* does not include the development of the axial row in sufficient detail to admit of a comparison. His earlier work (1918) on *Pallavicinia Lyellii* is more satisfactory in respect to the archegonium. In this species the axial cell is said to divide into a primary neck-canal cell and a primary ventral cell.

> The development of the axial row [neck-canal row] usually precedes the division of the primary ventral cell, although frequently mitoses can be seen in the neck cells after the formation of the ventral canal cell and egg. In most cases about ten neck-canal cells were

seen; sometimes, however, as many as eighteen are formed. The primary ventral cell, by a transverse division, produces a ventral canal cell and egg which are almost equal in size.

The ventral canal cell disintegrates very soon after being formed, and its disintegration is followed by that of the neck-canal row.

The most satisfactory account of archegonial development in any of the Jungermanniales (not including Sphaerocarpales) is that of Grün (1914) for *Treubia insignis*. Here the mother cell of the axial row divides, as in probably all the Hepaticae, into a central cell and a neck-canal mother cell.

> From the neck-canal mother cell there next arise successively four neck-canal cells. These undergo in the course of development a doubling to eight cells. These latter again divide so that we find in the fully grown archegonium sixteen neck-canal cells. During the formation of the eight neck-canal cells a division is likewise observed in the ventral tier of cells, that is, in the secondary central cell which has previously been changing slowly into a spherical form. This [division of the central cell] results in the formation of a smaller ventral canal cell and a considerably larger spherical egg.

Of the Sphaerocarpales, *Geothallus tuberosus* has been studied by Campbell (1896), *Sphaerocarpos texanus* by Miss Rojas (1918), and *S. Donnellii* by Miss Hartman (1918). All three are of the Riccia type so far as concerns the development of the axial row. The mother cell of the axial row divides into a central cell and a neck-canal mother cell. The latter, by two successive divisions, forms four neck-canal cells, these divisions being followed by an unequal division of the central cell to form the egg and ventral canal cell (the consecutive order of divisions is not stated for Geothallus).

The present state of our knowledge does not justify an extended generalization on the development of the axial row in the liverworts. In all the Hepaticae described (not including the Anthocerotales, which differ only slightly), this row develops from a primary axial cell which divides first into a central cell and a neck-canal mother cell. The latter by successive divisions gives rise to the neck-canal cells whose number is usually a power of two (occasional exceptions as to number in Riccardia, Pellia, Reboulia, and doubtless other forms). In the Jungermanniales studied the division of the central cell precedes the last division of the neck-canal cells (with possible exceptions in Pellia, according to Hutchinson, and in Pallavicinia, according to Haupt). In the Sphaerocarpales there are only two successive divisions of the neck-canal mother cell and its daughter cells, and these are completed before the division of the central cell. In the Marchantiales, the division of the central cell may be followed by one or more divisions in the neck-canal cells, as in Reboulia (Haupt, 1921), or it may occur after the last of these divisions, as in Riccia (Janczewski, Garber), or both conditions may be found in the same species, as in Marchantia (Durand). Which of these conditions is the more primitive, one can only conjecture. If we assume that the condition characteristic of the Jungermanniales is the more primitive, we might conclude that the last division of the neck-canal cells has been suppressed in the Sphaerocarpales and in

the simpler Marchantiales. A possible basis for such an assumption is afforded by Marchantia (Durand), in which the four neck-canal cells sometimes become binucleate as though preparing for this suppressed division; but this fact might also be interpreted in a converse manner to support the assumption that the condition in the Sphaerocarpales is the more primitive one, and that the Jungermanniales and the higher Marchantiales have acquired the habit of additional divisions in the neck-canal cells. It has not been shown whether or not environmental conditions may affect the number of divisions in the neck-canal cells. Hutchinson found only half as many neck-canal cells in *Pellia epiphylla* as did Janczewski. This reduction may conceivably have resulted from a suppression of the last division due to the influence of the environment in which his plants had grown.

Non-functional Archegonia

Abnormal sex organs are generally recognized as occurring frequently in the Bryophyta, and Florin (1918) has described several abnormal archegonia in *Riccardia pinguis*. One of these is an archegonium with a single egg and a double canal row. In my material I have found one archegonium which may be considered a complementary case. In this archegonium there are two eggs and two ventral canal cells, but only one row of neck-canal cells (fig. 38, Pl. XVIII). One egg and one ventral canal cell form the usual part of an apparently normal axial row. The additional egg and ventral canal cell appear slightly lower down in the archegonium and are separated from those of the complete axial row by a film which seems to be a cell wall. This archegonium is considerably more massive than those of the usual type, and a rift appears between the wall proper and the cells immediately above the supernumerary ventral canal cell. I have not been able to determine how far this rift extends around the column of cells above the ventral canal cell. The cells of this column have angular walls and numerous plastids and present the appearance characteristic of cells of the archegonial wall, with which the column is continuous above.

In the locations from which my material was collected Riccardia produces large numbers of archegonia, of which only a small percentage function in reproduction. It is apparent from the sections that a large percentage of the mature archegonia in the material collected were not capable of so functioning. In many cases it appears that disintegration has begun in the egg and the ventral canal cell before the maturity and normal disintegration of the neck-canal cells (figs. 36, 37, Pl. XVIII; figs. 42–46, Pl. XIX). Those in which this disintegration appears to be well advanced stain deeply with safranin although the cytoplasm of the neck-canal cells does not take this stain (figs. 36, 37, Pl. XVIII; and figs. 42, 45, 46, Pl. XIX). This premature disintegration of the egg and ventral canal cell is generally accompanied by a marked shrinkage of these cells, and the later swelling of the neck-canal cells sometimes causes the latter to push down into the venter (fig. 44).

Another type of disintegration frequently found is apparently due to the failure of the egg to be fertilized. Although the time during which the egg is capable of functioning as a gamete is probably short, the process of disintegration seems to be relatively slow. One sometimes finds the egg nucleus easily recognizable after the protoplasts have disappeared from the other cells of the archegonium. In the case shown in figure 40, the cytoplasm of the egg is represented only by a droplet of non-staining liquid in which are suspended globules of deeply staining material, while the nuclear membrane and nucleolus seem to be intact. Figure 39 represents an early stage of disintegration in which the cytoplasm still shows some of its alveolar structure but takes the basic stain.

Disintegration is also found to occur even after the first segmentation of the fertilized egg. Figure 41 represents a disintegrating embryo of two cells in an archegonium from the cells of whose wall all protoplasmic contents have disappeared. In this case the conditions inhibiting normal development probably obtained before the segmentation of the zygote, which has not elongated into the typical haustorial cell and epibasal cell (fig. 14, Pl. XVI; see also Miss Clapp's fig. 34).

The striking resemblance of many of these disintegrating cells to the figures shown by Florin and Miss Clapp arouses some doubt as to whether the cells figured by these workers were in all cases functional eggs. I have not found a case exactly similar to the one shown by Florin in his figure 1, but such a case would be comparable to some of those figured in the present paper. He says:

> In one archegonium [his fig. 1] I found four cells in a row, all morphologically and probably physiologically equivalent and supplied with large nuclei and deeply staining cytoplasm. They had also the appearance normally possessed only by the egg.

In view of the conditions found in my material, I should rather suspect that the archegonium represented in his figure is one in which disintegration had begun simultaneously in all four of the axial cells of about the stage shown in my figures 27 and 28, Plate XVII. Certainly the cells figured show little resemblance to the functional egg (fig. 33, Pl. XVIII). Miss Clapp's figure 33 is much more nearly typical, but probably represents an early stage of disintegration due to the lack of fertilization such as is represented in the present paper by figure 39, Plate XIX.

No attempt has been made to follow the sequence of cell divisions in the growth of the wall of the archegonium. These divisions appear to be quite irregular and frequent until the time of fertilization and afterward.

The mature archegonium is a massive structure, and, as described by Miss Clapp, its wall, except for a very small terminal portion, has two layers of cells (figs. 30–33, 35, 36, Pl. XVIII; figs. 42–50, Pl. XIX; Miss Clapp's figs. 32, 33). The neck of the archegonium is not made up of five longitudinal rows of cells as described by Janczewski for the Jungermanniales

studied by him, but shows in transverse section many more than that number. Figure 47 shows a cross section of the neck of a nearly mature archegonium at a distance of about 20 μ from its tip. Figure 48 shows a similar section of the same archegonium at a distance of about 50 μ from the tip. Figure 49 shows the cross section of the same archegonium just above the venter and about 80 μ from the tip. Figure 50 shows the section through the egg and venter at a distance of about 110 μ from the terminal end.

The development of the embryo seems to furnish a stimulus to very rapid growth and division in the cells of the venter as well as in those of the thallus immediately below (figs. 14–17, Pl. XVI). This results in the early formation of a massive "calyptra," of which only the upper portion has been derived from the archegonium (figs. 17, 18; see also MacVicar, 1912, p. 50, fig. 1). The cells of the thallus in close proximity to the young embryo contain a large number of chloroplasts and are doubtless active in metabolism. As observed by Miss Clapp, rhizoids arise from the basal portion of the "calyptra" and probably help to supply water and mineral nutrients for the increased metabolic activity.

According to Miss Clapp, the first segmentation of the fertilized egg is tranverse and divides the zygote into a hypobasal cell, which elongates into a haustorium, and an epibasal cell which gives rise to the embryo proper. Only three two-celled embryos have been found in my preparations, and none of these appears to have been alive or growing when fixed. The one shown in figure 41, Plate XIX, was sectioned obliquely, but it is obvious that it was in a degenerating condition; it had not elongated, the cytoplasm of the cells shows no definite organization, and all the protoplasmic content has disappeared from the cells of the wall of the archegonium. Figure 14, Plate XVI, shows another two-celled embryo whose haustorium is fairly well developed, but whose epibasal cell had apparently begun to disintegrate; the nucleus is scarcely recognizable, and the cytoplasm is disorganized. The third two-celled embryo, not figured, is intermediate between these two, both as to the amount of elongation and as to the degree of preservation of its protoplasmic content.

The youngest apparently normal embryo found is shown in figure 15, Plate XVI; it consists of three cells, a haustorium of one cell and the embryo proper of two cells. In this case the division of the epibasal cell was in a plane at right angles to the first division of the zygote. Figure 16 represents a longitudinal section of a nine-celled embyro; the embryo proper consists of a regular octant, of which two cells adjacent to the haustorium are in telophase. Figure 17 shows a later stage, in which the haustorium has reached its full size and in which the capsule can be distinguished from the seta. Figure 18 shows in outline the mature sporophyte within the massive "calyptra."

Miss Clapp describes the first three divisions in the zygote as horizontal, resulting in the formation of a filament of four cells, including the haus-

torium. My observations on these early stages do not accord with hers, but are too fragmentary to justify a questioning of her results.

Summary

1. *Riccardia pinguis* is a cosmopolitan species whose morphological features very somewhat under different environmental conditions.

2. The species is probable dioecious, but the male and female thalli are distinguishable only by their reproductive branches or by the presence or absence of sporophytes.

3. The spores when discharged from the capsule do not ordinarily adhere in tetrads, but the tetrads may be preserved by proper manipulation.

4. The growth of the thallus is apical, by means of a "two-faced" apical cell which gives rise to segments alternately from its right and left cutting faces.

5. The early divisions of the primary segment of the apical cell follow the scheme of Leitgeb (1877).

6. The axial row of the archegonium develops from a mother cell which divides to form a central cell and a neck-canal mother cell; the latter divides once, increasing the axial row to three penultimate cells which divide in acropetal succession forming ultimately an egg, a ventral canal cell, and four neck-canal cells. Occasionally, the number of penultimate cells is four instead of three and the ultimate number of neck-canal cells six instead of four.

7. Disintegrating archegonia are numerous in the material used for this study; the egg and ventral canal cell frequently break down before the maturity and disintegration of the neck-canal cells.

8. The young embryos found in this material show an order of division slightly different from that reported by Miss Clapp (1912).

9. A massive "calyptra" is formed around the sporophyte by the rapid growth and division of the cells of the venter and of the cells of the thallus immediately below the archegonium.

I wish to acknowledge my gratitude and indebtedness to Dr. W. N. Steil, who gave valuable assistance in procuring material; to Mr. Severin Rapp of Sanford, Florida, who supplied living plants from his locality; to Professor A. W. Evans of Yale University, who confirmed the identification of all material used and gave valuable suggestions; and especially to Professor C. E. Allen, under whose guidance these studies were made.

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EXPLANATION OF PLATES

All figures were drawn from stained sections with the aid of an Abbé camera lucida, using Leitz objectives and oculars, and were reduced in reproduction to the magnifications indicated; Plate XVI being reduced three fifths, Plates XVII–XIX one half.

Plate XVI

Fig. 1. Horizontal section of a part of the apical region of the thallus showing the apical cell (*a*) in process of division. × 160.

Fig. 2. Vertical longitudinal section of secondary segments (*b'* and *c*) formed by the division of a primary segment; serial section adjacent to and following the one shown in figure 3. × 160.

Fig. 3. Median vertical longitudinal section of the apical region of a vegetative branch of the thallus showing apical cell (*a*). × 160.

Figs. 4–8. Successive serial transverse sections of the apical cell (*a*), and of the adjacent cells in a vegetative branch; *b'* and *c* at the right in figure 4 probably represent the anterior ends of cells formed from the youngest segment of the apical cell; *c*, *d*, *e*, and *e'* at the left in the same figure probably represent the anterior ends of the forward tier of cells formed from the second youngest segment. × 160.

FIG. 9. Vertical longitudinal section through the primary segment (*b*) in process of its first division; serial section adjacent to and preceding the one shown in figure 10. Greenhouse plant of Florida stock. × 160.

FIG. 10. Median vertical longitudinal section of apical end of vegetative branch showing apical cell (*a*). Greenhouse plant of Florida stock. × 160.

FIG. 11. Vertical transverse section of apical cell (*a*, *b*) in process of division; *b* represents the portion which would have become the segment. Greenhouse plant of Florida stock. × 160.

FIGS. 12, 13. Successive vertical longitudinal sections of a young archegonial branch; figure 12 shows the apical cell *a*, figure 13 the secondary segments *b'* and *c* and a three-celled archegonium (♀). × 230.

FIG. 14. Longitudinal section of archegonium and two-celled embryo (probably not functional); reconstructed from two adjacent sections. × 230.

FIG. 15. Longitudinal section of archegonium and three-celled embryo; reconstructed from four serial sections. × 230.

FIG. 16. Longitudinal section of nine-celled embryo and gametophyte tissue enlarging to form the massive calyptra; *V*, ventral surface of thallus; *D*, dorsal surface; reconstructed from four serial sections. × 116.

FIG. 17. Longitudinal section of young calyptra and young sporophyte; *V*, ventral surface of thallus; *D*, dorsal surface; reconstructed from three serial sections. × 116.

FIG. 18. Outline of longitudinal section of mature calyptra and nearly mature sporophyte; reconstructed from two sections not adjacent. Greenhouse plant of Wisconsin stock grown in 1920. × 20.

PLATE XVII

Longitudinal sections of archegonia.

FIG. 19. Very young archegonium showing the mother cell of the axial row. × 950.

FIG. 20. Mother cell of axial row in early prophase of division. × 950.

FIG. 21. Mother cell of axial row with division almost completed. × 950.

FIG. 22. Central cell and neck-canal mother cell formed by first division. × 950.

FIG. 23. First division of neck-canal mother cell in progress. × 950.

FIG. 24. Axial row of three penultimate cells, the central cell and two neck-canal cells. × 618.

FIG. 25. Early prophase of division of the central cell. × 618.

FIG. 26. Late telophase of division of the central cell. × 375.

FIGS. 27, 28. Axial row of four cells—egg, ventral canal cell, and two penultimate neck-canal cells. × 375.

PLATE XVIII

FIG. 29. Lower penultimate neck-canal cell in process of division. × 375.

FIG. 30. Axial row consisting of five cells—egg, ventral canal cell, two ultimate neck-canal cells, and the upper penultimate neck-canal cell, the latter in prophase of division. × 375.

FIG. 31. Mature axial row consisting of egg, ventral canal cell, and two binucleate neck-canal cells; reconstructed from three serial sections. × 375.

FIG. 32. Axial row consisting of egg, ventral canal cell, and one penultimate neck-canal cell; reconstructed from two sections. × 375.

FIG. 33. Mature archegonium with egg ready for fertilization, antherozoid present in venter; reconstructed from two sections. × 375.

FIG. 34. Axial row consisting of egg, ventral canal cell, and three penultimate neck-canal cells; reconstructed from four serial sections. × 375.

FIG. 35. Similar to figure 34, except probably a little older; reconstructed from two sections. × 375.

FIG. 36. Axial row consisting of egg, ventral canal cell, one binucleate neck-canal cell, and two penultimate neck-canal cells in process of division; egg and ventral canal cell disintegrating; reconstructed from three serial sections. × 375.

FIG. 37. Mature axial row with three binucleate neck-canal cells, all markedly shrunken; egg and ventral canal cell badly disorganized. × 375.

FIG. 38. Archegonium with two eggs, two ventral canal cells, and one neck-canal row; reconstructed from three serial sections. × 375.

PLATE XIX

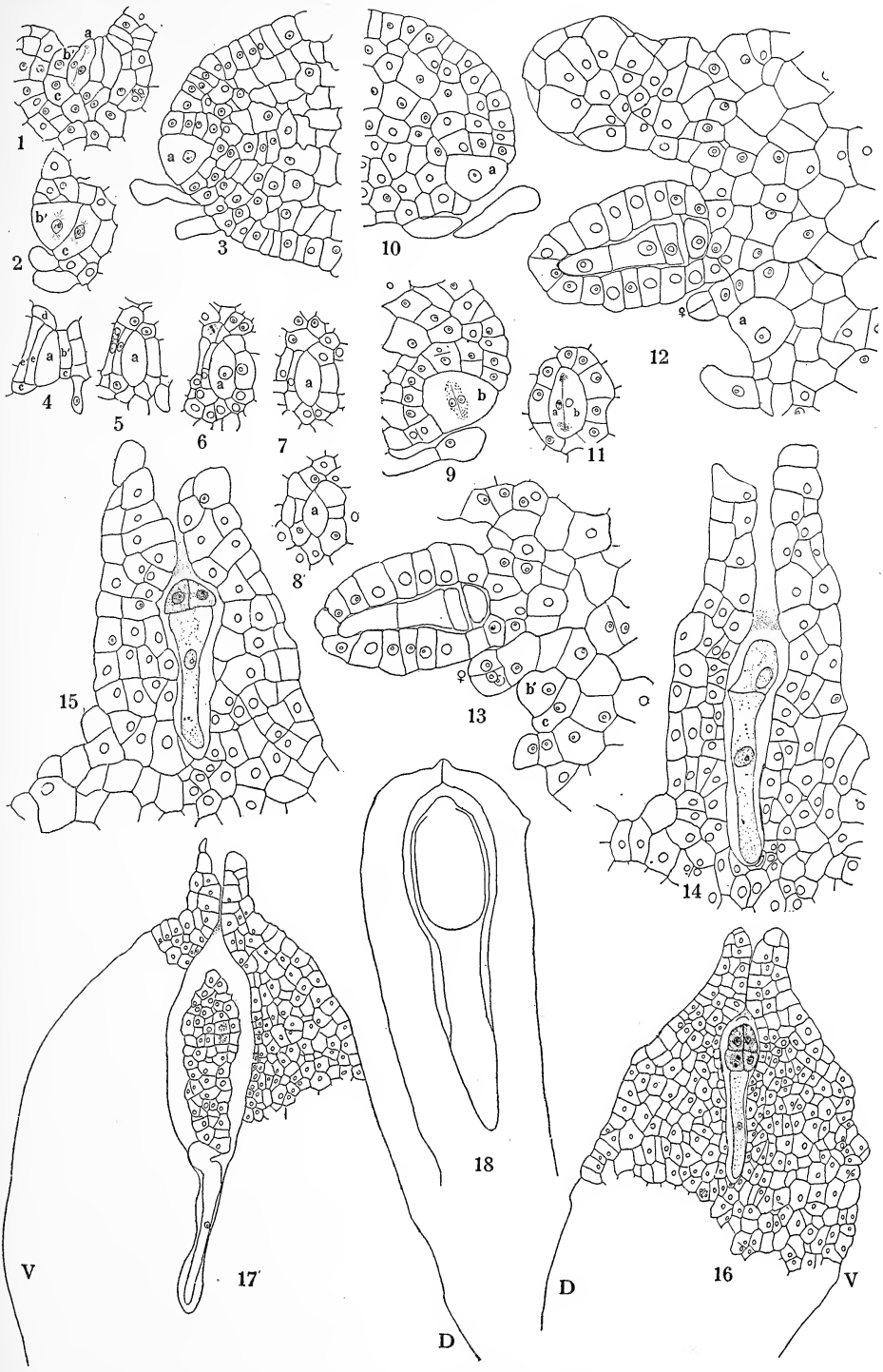
FIG. 39. Longitudinal section of archegonium showing egg in early stage of disintegration due to failure to be fertilized. × 375.

FIG. 40. Archegonium showing late stage of disintegration; no protoplasm visible in cells of wall but nucleus of egg still recognizable. × 375.

FIG. 41. Oblique section of archegonium and two-celled degenerating embryo; no protoplasm in cells of archegonial wall. × 375.

FIGS. 42–46. Premature disintegration of egg and ventral canal cell; figure 45 reconstructed from two sections. × 375.

FIGS. 47–50. Transverse sections of a mature archegonium; figure 47, third serial section from terminal end; figure 48, sixth serial section; figure 49, ninth serial section, just above the venter; figure 50, twelfth serial section, through venter and egg. × 200.



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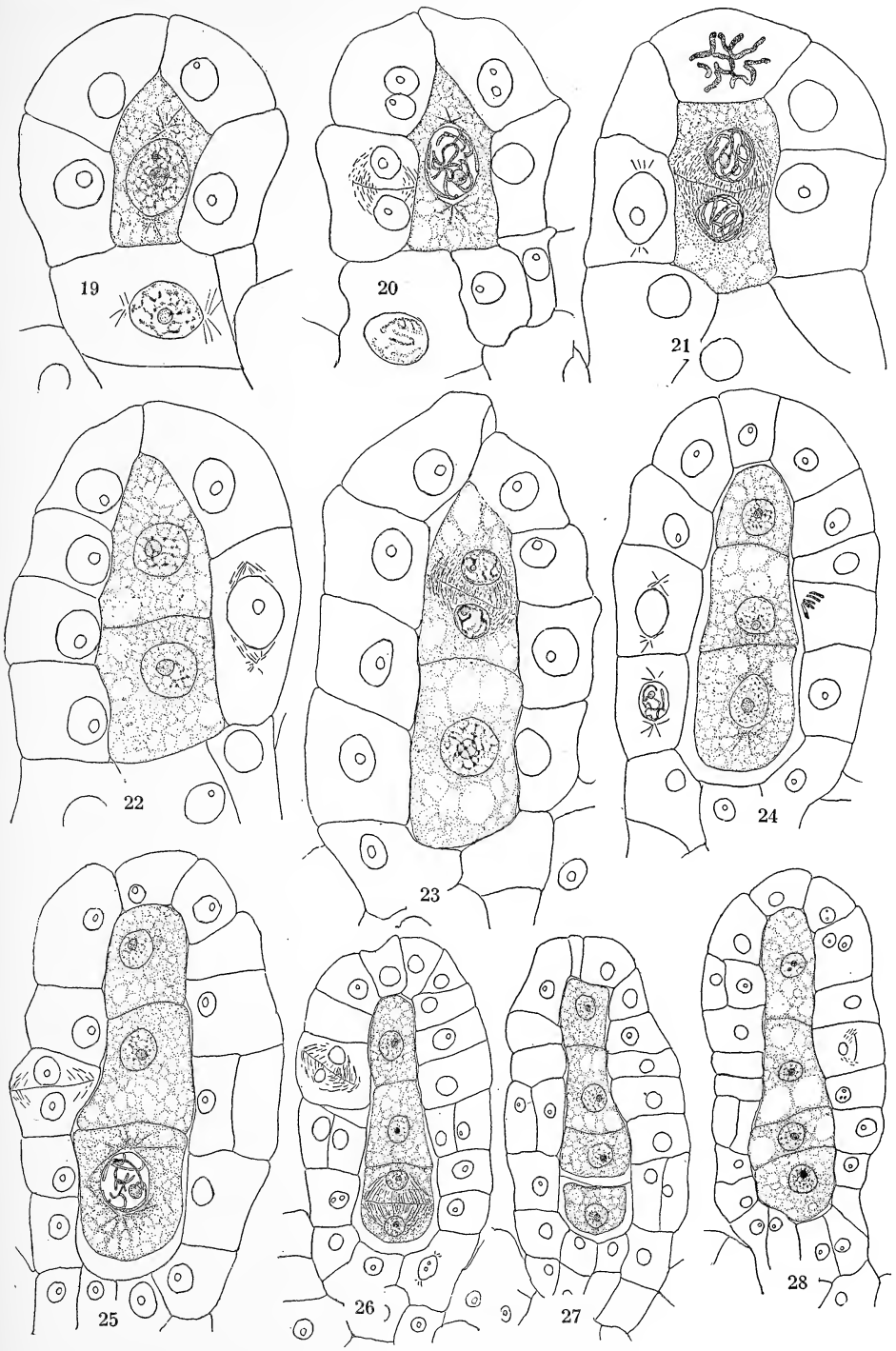

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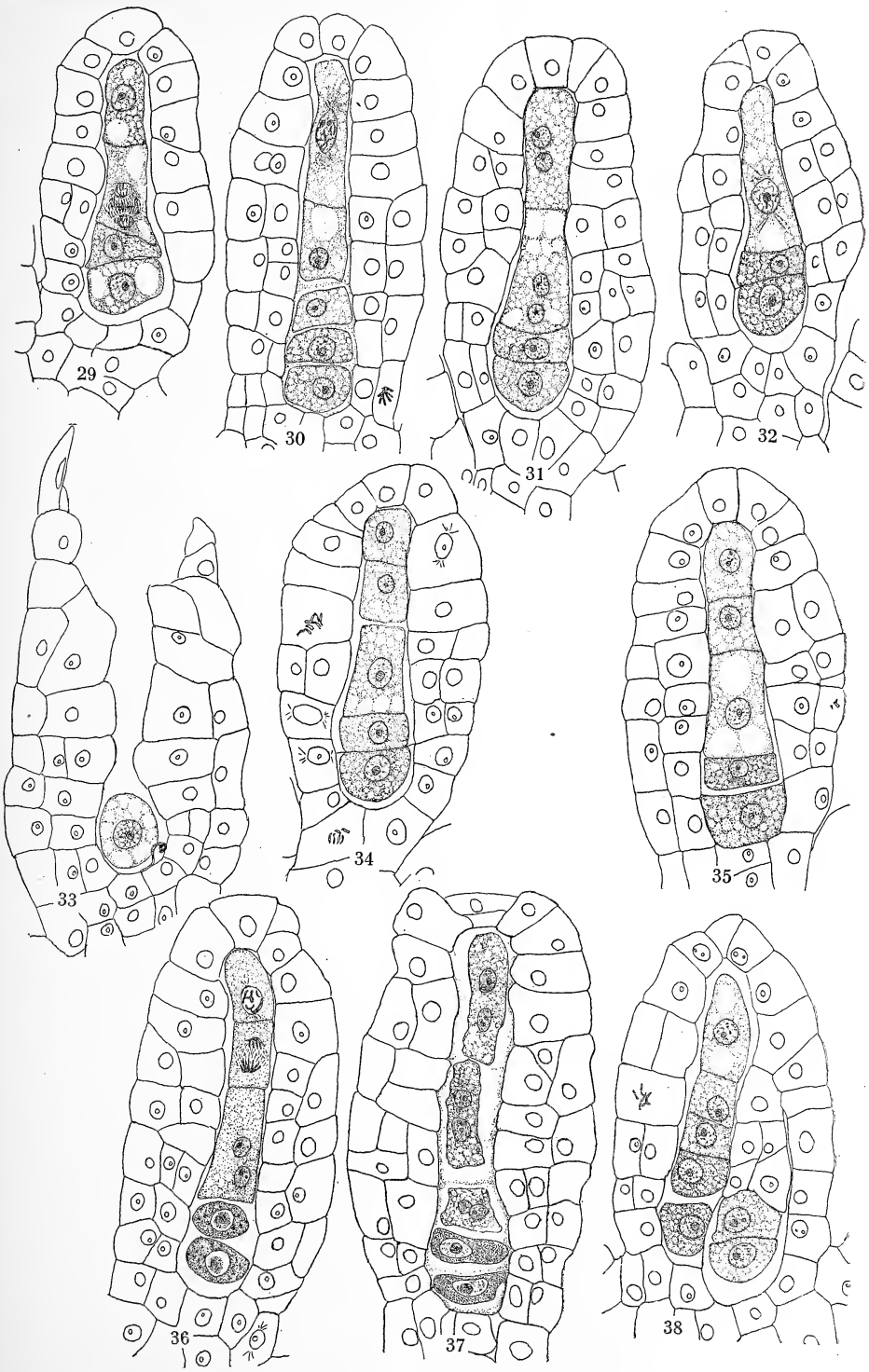

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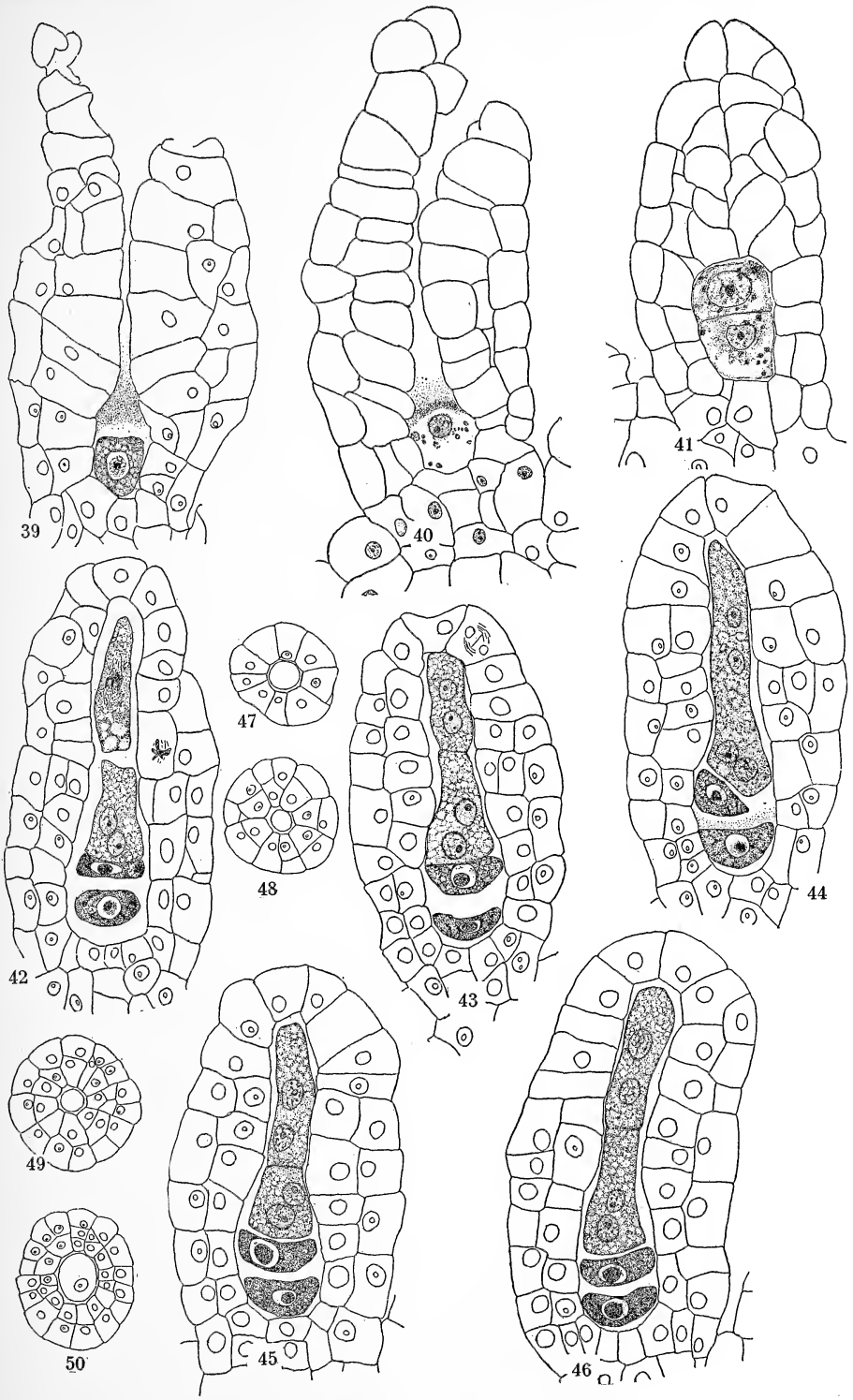

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PECTINASE IN THE SPORES OF RHIZOPUS

J. L. WEIMER AND L. L. HARTER

(Received for publication April 28, 1922)

Kopeloff and Kopeloff[1] showed that the deterioration of cane sugar depends in part at least on the hydrolyzing power of an enzym which is contained in the spores of such fungi as *Aspergillus niger* Van Tiegh., *A. sydowi* Bain., and to a lesser extent *Penicillium expansum* Link and *Aspergillus flavus* Link. Harter and Weimer[2] also demonstrated that the spores of *Rhizopus tritici* Saito and *R. nigricans* Ehrb. contain an enzym capable of hydrolyzing starch to reducing sugars.

Pectinase production has been the subject of extensive researches by Harter and Weimer.[3] They found that this enzym is produced by several species of Rhizopus; that some of it is retained in the mycelium, and that a portion is exuded into the substratum. In their experiments, raw sweet-potato discs immersed in the substratum or in a watery extract of the mycelium were macerated so that coherence of the cells was entirely lost. The dissolution of the middle lamellae was in every way analogous to that produced by the action of the fungus itself in the host tissue. The enzym was found produced most abundantly in cultures two to three days old. They proved, moreover, that the enzym is secreted very early in the germination of the spore. In fact, maceration of raw sweet-potato discs took place slowly when suspended in a nutrient solution in which spores of *Rhizopus tritici* had been placed for only eight hours. At the end of this time a considerable percentage of the spores had germinated, the germ tubes varying in length from one to ten times the diameter of the spores.

The writers, after having shown that pectinase is exuded into the solution on which the fungus grew and that it is present in the mycelium, undertook to demonstrate the presence or absence of the enzym in the ungerminated spores.

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Methods of Experimentation

Two species of Rhizopus, *R. tritici* and *R. nigricans*, were employed. These two species were selected because among the parasitic species they represent the two extremes in the amount of pectinase produced. *Rhizopus tritici*, under the conditions of the writers' previous experiments, secreted a very active macerating enzym while that secreted by *R. nigricans* was much less active.

The spores were obtained in the following manner: Several 2-liter flasks containing about 500 cc. of sweet-potato decoction were inoculated with the organisms under investigation. At the end of ten days or two weeks spores were abundantly produced. The fungous felt was then carefully lifted from the flasks, the lower side of the felt was held under the tap for a few moments to wash away the decoction, and then the felt was floated, the upper side down, in a vessel of distilled water. By gently agitating the fungous mat a considerable quantity though not all of the spores were removed. The spore suspension was then filtered through a good grade of muslin, about 27 threads to the centimeter, to remove any fungous threads that might have broken off during the process of removing the spores. At this stage a microscopic examination of the suspension was made, and if any bits of mycelium were found in the solution it was either filtered again or discarded. When the suspension was free of fungous *débris* the spores were caught on a number 2 chemically prepared Whatman filter paper. The spores were then washed from the filter paper with acetone. They were exposed to the acetone for 10 minutes, then caught on a tarred filter paper, and finally treated with ether by pouring the latter on to them on the filter paper. The filter paper was then removed from the funnel, straightened out, and stored away in any suitable container until required for use. Tests have shown that spores so treated will not germinate.

After the dry weight of the spores was obtained, their macerating action on raw sweet-potato discs was determined by immersing the latter in a water suspension of the spores. At this point no attempt was made to separate the spores from the paper, the latter being included in the system. The amount of water used was determined by the weight of the spores. Ten cubic centimeters of water were used for every 0.10 gram of spores. Since it was impossible by the methods employed to obtain a definite weight of spores, the raw discs were exposed to a spore suspension of fairly uniform density by this adjustment of the relationship of the water and spores.

Experimental Data

Several experiments were conducted with the spores of both *Rhizopus tritici* and *R. nigricans*. In all cases except a few of the preliminary ones, the actual weight of the spores was determined. In every case one control

was held in which the spore suspension was heated in order to inactivate the enzym. Maceration was carried out at a temperature of 35° C. A little toluol was added to each flask to prevent the growth of contaminating bacteria.

The enzym was relatively dilute, so that maceration progressed rather slowly. No maceration occurred in the control flasks. In the other flasks the discs became somewhat crisp at first but later became flaccid. This stage was then followed by a dissolution of the middle lamellae, which was typical in every respect of that produced by a water extract of the mycelium or by the solution on which the fungus grew.

The rate of the maceration by the enzym produced by the spores of these two species differed considerably. The action by *R. tritici* was relatively rapid, the discs being completely macerated in 24 to 48 hours. *Rhizopus nigricans*, on the other hand, acted very slowly, and in some cases no maceration took place in 72 hours. The writers have found also that the enzym per unit volume of solution or per unit weight of mycelium is much weaker in *R. nigricans* than in *R. tritici*. Without going further into the details of these experiments or their results, the evidence conclusively shows that the spores of both species contain an enzym which is able to cause disintegration of the middle lamellae of raw sweet potatoes.

It is very likely that this enzym plays an important rôle in the early nutrition of the fungus and that it may be a factor in the initial infection of some of its hosts.

Summary

The spores of *Rhizopus nigricans* and *R. tritici* both contain an enzym, pectinase, which is capable of dissolving the middle lamellae of raw sweet potatoes. The rate of maceration by the spores of *R. nigricans* is relatively much slower than that produced by the spores of *R. tritici* when the concentration of the spores by weight is the same.

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THE CHROMOSOMES OF *RICCARDIA PINGUIS*

AMOS M. SHOWALTER

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This study of the chromosomes of *Riccardia pinguis* (L.) S. F. Gray was made with reference primarily to possible sex differences. The material used was collected August 11 and 12, 1921, in the swamp prairie bordering Lake Waubesa, near Madison, Wisconsin. The methods of fixation, staining, etc., have been reported in a previous paper (Showalter, 1923) and need not be repeated here. This material was supplemented with greenhouse plants grown from Florida and Wisconsin stock.

My choice of this plant for such a study was due in part to a suggestion of Dr. W. N. Steil, who had observed that the male plants are sometimes noticeably smaller than the female, but my observations in field and cultures have convinced me that there is no marked sexual dimorphism in this species.

Division figures are fairly frequent in the massive embryonic tissue at the growing ends of the thallus and in the young sex organs. The chromosomes are relatively large and are easily stained so that they stand out in brilliant color contrast to the rest of the cell contents. The spermatogenous cells of the antheridium, however, are less favorable for counts of the chromosomes because the cells are small (except in very young antheridia) and division figures are consequently crowded, so that the chromosomes of one cell are not easily distinguished with certainty from those of the adjacent cells.

In the cells of the embryonic tissue of both male and female plants and in those of the archegonium, the chromosomes are frequently well spread out on the spindle at the equatorial plate stage and in polar view are especially favorable for study. I have studied a large number of division figures in the plants from the region of Madison, and the evidence seems conclusive that the haploid number of chromosomes is ten (figs. 1–18, Pl. XX). Farmer (1905), in studying the reduction divisions, reported casually that the number "seems to be eleven for the species in question." Occasionally a small spherical body which stains like a chromosome is found near or among the chomosomes (figs. 2, 11, 12). I have not traced the behavior of this body, but there is little reason to suspect that it may be chromosomal in nature or origin. It may possibly be a fragment or vestige of the nucleolus.

The chromosomes are relatively smooth, rod-shaped structures, fairly uniform in thickness and variously bent (figs. 1–19). They differ somewhat in size, but only one, the smallest, is distinguishable with certainty in any large number of cases. The chromosomes which appear in sporo-

phytic divisions have the same general appearance as have those seen in the thallus, though, of course, in the double number (fig. 19).

I have not studied exhaustively the chromosomes of the plants received from Florida, but the few dozen division figures examined have convinced me that the number and relative sizes of the chromosomes are the same as those of plants from the vicinity of Madison (figs. 20–24). In two or three division figures from the Florida plants there appear to be eleven chromosomes (fig. 21), but this appearance may be due to a part of one chromosome having been displaced by the microtome knife.

Previous investigators of the morphology of this species agree that it is strictly dioecious, but this conclusion seems to be based upon field observations and not, so far as I have been able to find, upon conclusive cultural experiments. My own attempts to grow the plant in culture have not been highly successful and have yielded no conclusive evidence, but in all cases observed, both in cultures and in the field, the male and female sex organs were produced on separate plants.

The comparative study of the chromosomes of the two sexes, as in the case of my earlier study of the chromosomes of Conocephalum (Showalter, 1921), has revealed no perceptible difference between the sexes. Exact micrometric measurements of the chromosomes have not been made, but the camera lucida drawings show no perceptible difference between the chromosomes of the male and those of the female plants (figs. 1–18). The evidence seems conclusive that there is in this species no such chromosome difference between the sexes as is found in Sphaerocarpos (Allen, 1917, 1919; Schacke, 1919).

I wish to express my gratitude to Professor C. E. Allen, under whose direction this study was made.

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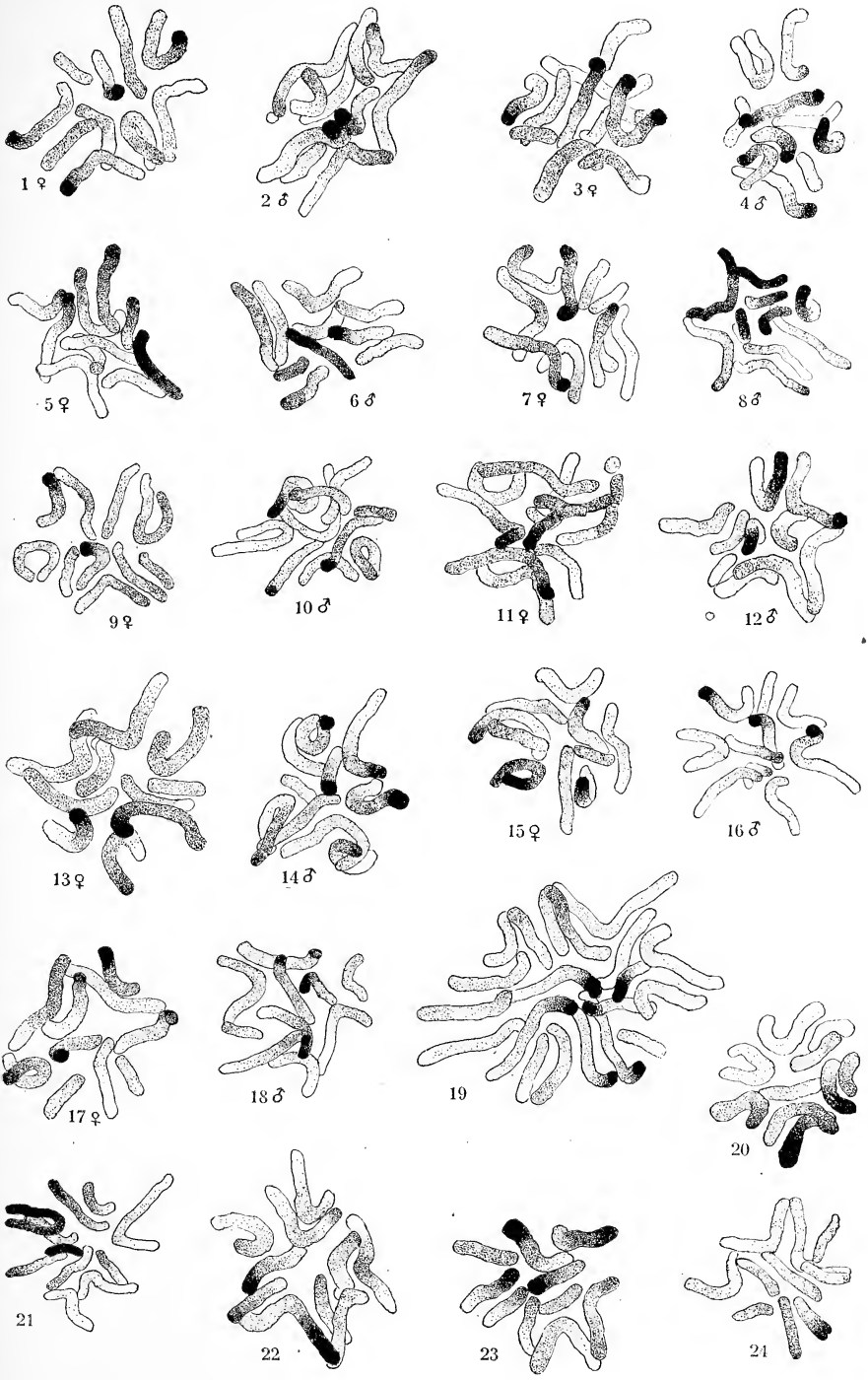
EXPLANATION OF PLATE XX

All figures were drawn with the aid of an Abbé camera lucida, using a Zeiss 2-mm. apochromatic objective N. A. 1.40, and compensating ocular 18, at a magnification of about 4,000 diameters; reduced in reproduction to about 2,650.

FIGS. 1–18. Chromosome groups, alternately from female and male plants (Wisconsin stock), as indicated by symbols: 1, from cell of dorsal surface layer of thallus; 2–8, from cells of interior of thallus; 9, from cell of wall of archegonium; 10, from cell of dorsal surface layer; 11, from cell of wall of archegonium; 12, from cell of interior of thallus; 13, from cell of papillate scale; 14, from cell of dorsal surface layer; 15, from cell of ventral surface layer; 16, from cell of interior of thallus; 17, from cell of dorsal surface layer; 18, from cell of ventral surface layer.

FIG. 19. Chromosomes in cell of capsule wall, from greenhouse culture of Wisconsin stock.

FIGS. 20–24. Chromosomes of greenhouse plants grown from Florida stock: 21, 22, from cells of interior of thallus; 23, from cell of ventral surface layer; 24, from cell of dorsal surface layer.



SHOWALTER: CHROMOSOMES OF RICCARDIA

AUSTRALASIAN BOTANICAL NOTES

II. VICTORIA, SOUTH AUSTRALIA, AND WEST AUSTRALIA

Douglas H. Campbell

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Victoria

Victoria, the smallest of the Australian States except Tasmania, has a rather more uniform climate, both as to temperature and rainfall, than most of Australia, and in this respect more nearly resembles certain regions of the north-temperate zone. Victoria occupies nine degrees of longitude and five of latitude, its southernmost point, Wilson's Promontory, extending just beyond the thirty-ninth parallel. In area Victoria is 87,884 square miles, a little more than the area of Kansas. The eastern portion, Gippsland, is a continuation of the coastal belt of New South Wales, and to the north of this is an elevated region, which is a continuation of the main mountain mass of New South Wales. Within Victoria this is known as the Victorian Alps. In the southwest there is an extensive basaltic plain of great fertility, one of the most productive regions in the commonwealth.

The northwest corner of the state is a continuation of the interior plains of New South Wales and South Australia, and like them is a region of scanty rainfall.

The heaviest rainfall is in parts of Gippsland, where some stations have over sixty inches, and nearly a third of the state has a rainfall exceeding thirty inches.

In these well-watered regions, especially in the mountainous parts of Gippsland, there is a heavy forest, mainly Eucalyptus. It is in the Gippsland forest that the giant among Australian trees is found. This is *Eucalyptus regnans*, which is a close rival in height of the Californian Sequoias.[1]

Victoria has proportionally a larger amount of land available for ordinary agriculture than any of the other states, and in consequence is more uniformly populated and looks more like the agricultural regions of Europe and America.

Melbourne is in many ways the finest of the Australian cities. Its broad, well-kept streets and handsome and substantial public buildings and business structures give the impression of a remarkably prosperous community.

[1] Maiden, in his sketch of the Australian flora (Federal Handbook, p. 204), states that the "official size" of the tallest Gippsland tree was 326 feet 1 inch height, and girth 25 feet 8 inches, six feet from the ground.

As in the other large cities, ample provision has been made for parks, including an uncommonly attractive and interesting botanical garden. Close to the gardens and the adjacent park lands is the river Yarra, which flows through the city. From the river an ample supply of water for the gardens is available, and this, together with good soil, gives the gardens a great advantage over those of Sydney, and this shows especially in the fine stretches of lawn and the luxuriant growth of many deciduous trees and shrubs which do not thrive in Sydney.

It is true that the colder winters of Melbourne are unfavorable to the growth of strictly tropical and many subtropical species; but this is counterbalanced by the much better growth of the plants of more temperate regions. Trees and shrubs from temperate America, Asia, and Europe do much better in Melbourne than in Sydney or Brisbane, and the same is true of the hardy bulbs and other spring flowers.

In September and October the gardens were looking very beautiful. The early flowering trees and shrubs, especially the Japanese cherries, double-flowering peaches, Magnolias, and Judas tree, were unusually good and made a brilliant display. Camellias and Indian Azaleas were also very fine, although the latter were perhaps not quite so luxuriant as in Sydney. There were also some good Rhododendrons. Where they were sheltered, several species of tree ferns and palms grew very well. A particularly satisfactory effect was attained in one place, where, under the shelter of large trees, there was a fine plantation of tree ferns and palms which looked very tropical. The tree ferns comprised several species of Cyathea, Dicksonia, and Alsophila, the finest being *A. excelsa* from Norfolk Island. The palms included the two native species, *Archontophoenix Cunninghamii* and *Livistona australis*, and the New Zealand *Rhopalostylis sapida*.

I was especially interested in an excellent collection of American trees, which seemed very much at home. These included very fine specimens of several oaks—*Quercus alba*, *Q. rubra*, *Q. coccinea*, and one or two others—Liquidambar, Taxodium, Robinia, Gleditschia, *Acer rubrum*, and *Cornus florida*. The latter was in flower, but the flowers were not abundant.

A large and well-labeled collection of Australian plants, including many of the very showy West Australian flowers, is an important feature of the garden. Among the more striking of these were flowering specimens of the great torch lilies (*Doryanthes excelsa*, *D. Palmerstoni*) and the beautiful scarlet gum, *Eucalyptus ficifolia*, from western Australia.

Because of the limited time at my disposal, I was able to make only two botanical excursions while in Victoria. Owing to its smaller size and more uniform climate, Victoria has a much smaller proportion of peculiar species than the larger states, comparatively few species being confined to it.

In the well-watered eastern sections there is a heavy forest, but only a small number of the Malayan types characteristic of the rain forests of Queensland and New South Wales extend into Victoria. In the extreme

north the palm *Livistona australis* is said to grow, and a few other subtropical genera occur; but for the most part the forest is composed of various species of Eucalyptus, of which the giants are *E. regnans* and *E. globulus*, the Tasmanian blue gum, so extensively planted in California and elsewhere.

On a former visit to Australia, in 1903, I made a brief trip to the Black Spur, in the Gippsland mountains, and saw some very fine specimens of the giant gums. This forest is a very beautiful one, as among the great trees in many places are groves of tall tree ferns. As much of the forest has been destroyed since my visit, one wonders whether any of the biggest of these trees are still standing.

The tree ferns are largely *Dicksonia antarctica*, but *Alsophila australis* also occurs. There are some interesting bryophytes also common in this region, notably the giant moss, *Dawsonia superba*, and the liverwort *Umbraculum flabellatum*.

A visit, in company with Professor Ewart of Melbourne, was made to this region, but at a considerably lower elevation; and as most of the forest had suffered greatly from fire, and the region as a whole seemed to be much dryer, the vegetation was decidedly less interesting. The forest trees were for the most part much smaller than on the Black Spur, and the ferns and liverworts were less abundant. Several pretty orchids were seen, and some interesting heaths and sundews. The latter, together with some of the orchids, were especially abundant where the ground had been recently burned over.

The most extensive excursion made in Victoria was to the National Park, in the extreme southern part. Wilson's Promontory, the southernmost point of Australia, has been reserved as a sanctuary for the native plants and animals, and is admirably placed for this purpose. One of the trustees of the park, Mr. Kershaw, accompanied me, and proved a most efficient and entertaining guide. The days spent in his company in the park are among the pleasantest recollections of my stay in Australia.

To reach the park one has to go about one hundred miles by rail, and then one may go part way by motor; but the rest of the way along the sea beach, for two or three hours, was done in a light buggy. On our return we crossed the inlet between the promontory and the mainland in a motor boat, but this is feasible only at high tide.

Going by land, one drives over roads that are by no means perfect—indeed, we were held for an hour or more by our motor being bogged—through low-lying land, much of it a bog. A good many flowers, expecially some pretty heaths, were noted, but no very careful observations of the flora were made. The shore drive was along a barren coast, with sand dunes as we approached the park.

The park comprises about 100,000 acres of mountain and forest, and occupies the greater portion of the peninsula. The isthmus connecting it with the mainland is only about seven miles wide, and across this neck of land there is a strong rabbit- and vermin-proof fence, which completely

shuts off the park and prevents either entrance to or egress from it. Here one may see at large, as nowhere else in Australia, many of the most remarkable members of Australia's strange and interesting fauna.

As we drove along the beach, great black swans rose from the lagoons and flew away, and in other places shore birds of various sorts ran across the beach at our approach. Within the park we found the swans and other water fowl very common. Of the birds, however, none were quite as strange as the emus, a flock of which frequented an open meadow not very far from the rest house where we stayed. Blue and scarlet parrots, and white cockatoos, as well as many other less striking birds, were not uncommon. Kangaroos, which were not at all rare, were seen, and the so-called native bear, a most amusing little animal, resembling exactly the "Teddy bears" of the toy shops.

The flora of the park, owing to the diversity of soil, moisture, and elevation, is a rich one. Along the coast are rocky promontories, extensive beaches, and sand dunes, on which were growing Mesembryanthemums and other dune plants, which were not collected.

About the rest house was open grassland, which was bordered by extensive, dense thickets of *Melaleuca* sp. covering large tracts of swamp land. The lower hill slopes were covered with a thicket of mixed shrubs, much like the chaparral of our Californian hillsides. The most conspicuous member of this scrub was *Leptospermum laevigatum*, then in full bloom and very pretty. The largest members of this association were small trees of *Casuarina* sp., and other shrubs were *Melaleuca* sp., *Acacia dealbata*, *Exocarpus* sp., and others.

The dryer ground was occupied by an open forest of gums, but with these were many Banksias, comprising three species, the commonest being *B. serrata* and *B. integrifolia*. Both of these were trees of fair size, and with them were associated Acacias, Casuarinas, and Hakeas.

The open spaces supported groves of grass trees (*Xanthorrhoea* sp.), their stout flower spikes rising eight or ten feet above the crown of drooping leaves. These thousands of flowering grass trees made one of the most peculiar sights that I remember. The very profuse flowering was attributed to the fact that the ground had been burned over the previous season. It was also on these burnt-over areas that there was the greatest profusion of orchids and sundews. In one such place we collected nearly twenty orchids, of which some were very attractive. These included several species of Caladenia, Glossodia, Diuris—especially showy bright yellow flowers—Pterostylis, and several small species which were not identified. Among the Caladenias were several of the curious species known popularly as "spider orchids," on account of the long slender extensions of the sepals. Hibbertia, Wahlenbergia, and several other pretty flowers were common, and one in particular was very handsome. This was an iridaceous plant, *Diplarrhena Moraea*, with large white flowers that suggested an orchid. The flower is somewhat zygomorphic, and there are but two perfect stamens.

One of the numerous rocky promontories was visited. Much of it was covered with a pretty dense growth of *Melaleuca ericifolia*, which bore a profusion of beautiful snow-white flowers, reminding me somewhat of some of the Californian species of Ceanothus. There were also quite extensive pure stands of *Casuarina* sp. forming small trees.

The exposed point of the peninsula was quite destitute of shrubs of any size, and the nearly flat summit was covered with the prevailing sandy soil in which scattered low shrubs and the usual herbaceous plants were growing.

The most extensive collecting trip was across the divide of the mountain backbone of the promontory. The trail is a pretty good one, and we made it on horseback. The first part of the trail rose steeply over bare rocks and flats of poor sandy soil, covered with dwarf Casuarinas and various low shrubs and herbs. There were some very pretty heaths, and a fine orchid. Thelymitra, with beautiful sky-blue flowers. After climbing for some time at about 1,000 feet elevation, the trail led through the forest, which grew moister and more luxuriant as we neared the summit of the pass.

Along the side of the trail were a good many pretty flowers, perhaps the finest being two bright scarlet heaths, *Epacris* spp. Victoria is expecially rich in these beautiful plants, which seem to prefer the cooler and moister conditions of eastern Victoria. Yellow Hibbertias and pink Tetratheca and Bauera were among the most abundant of these flowers along the trail.

As soon as the divide was passed the effects of the greater moisture on the windward side were apparent, and, as the trail descended, the increasing abundance of mosses and ferns gave evidence of the increasing moisture. Tree ferns began to be abundant in the gullies, and fine specimens of *Todea barbara* were seen. The banks and the rocky beds of the streams showed a rich growth of mosses and liverworts, among them the fine moss, *Dawsonia superba*, collected long ago at the Black Spur, and the beautiful liverwort *Hymenophytum* (*Umbraculum*) *flabellatum*.

There was not time to go to the bottom of the trail, where there are to be seen *Eucalyptus globulus* and the evergreen beech, *Nothofagus Cunninghamii*. Both of these are common Tasmanian species.

At the entrance to the park there are extensive sand dunes, which have been planted with "Marram grass" (*Ammophila arundinacea*), which seems to be very efficacious in holding the shifting sand.

I was unable to visit the Cape Oltway district in southwestern Victoria, where there are extensive forests of blue gum and beech (*Nothofagus Cunninghamii*).

South Australia

A large part of South Australia is desert, and the flora is less rich than that of the neighboring states, Victoria and West Australia. As my time was limited, I was able to obtain only the most superficial acquaintance with the flora of this state. South Australia lacks high mountains, the Mount Lofty range near Adelaide scarcely exceeding 3,000 feet altitude,

and this region receives a fair amount of rain; but out of a total area of 380,070 square miles in the state, over 300,000 receive less than ten inches of rain annually.

The region about Adelaide, the principal city, seems to be a fertile one, and when I saw it, after the abundant rains of last year, the young crops of grain and hay and the flowering orchards in the Mount Lofty district gave promise of an excellent harvest. There was the typical Eucalyptus forest, with Acacias and Casuarinas as undergrowth, and in some districts stands of the cypress-like *Callitris* sp.

Along the railway line were in many places masses of the European gorse and broom, which seemed to be throughly naturalized, and, as they were in full flower, they made a brilliant show. In this region, as elsewhere in Australia, a number of South African plants have become naturalized and in some cases are troublesome weeds. The "Cape weed," *Cryptostemma Calendulacea*, covered several of the fields with a solid carpet of pale yellow flowers, and the bright yellow *Oxalis cernua* was extremely abundant in many places. Sometimes in low ground the common calla lily could be seen, and several of the showy Iridaceae of South Africa, Sparaxis, Watsonia, and Homeria, were seen apparently quite naturalized. The latter is said to be poisonous and is regarded as a pernicious weed. At one place, not far from Adelaide, the cardoon (*Cynara Cardunculus*) was very abundant, but it was not noted elsewhere.

Adelaide is most attractively laid out, with a fine park along the river which traverses the town, and a good botanical garden. At the time of my visit, in September, the flowering deciduous trees and shrubs were at their best. Lilacs were in full bloom, and especially beautiful were the double flowered peaches—pink, white, or crimson, which were freely planted in the park and botanical gardens. Unusually large individuals of the Judas tree were also seen.

Of evergreen trees I noted several fine specimens of the redwood and the Californian bay tree (Umbellularia) and a gigantic specimen of the European *Arbutus unedo.* A remarkably fine specimen of *Araucaria imbricata* was a feature of the garden. I was much interested in an extensive collection of South African Iridaceae: Ixia, Sparaxis, Tritonia, and Freesia. The Cape bulbs, as might be expected, do remarkably well in Australia and Northern New Zealand. In the United States, except in the warmer parts of the Pacific Coast, these beautiful plants must be grown under glass.

Western Australia

Western Australia, from the scenic standpoint, is much inferior to the eastern coastal regions of Australia, as it is largely an extremely arid region and the mountains are low and not at all striking in appearance. The extreme southwest corner, however, has a fairly abundant rainfall, and the coastal portions of this area support a heavy growth of giant eucalypts.

The northern coast, with a tropical climate, has in some portions good summer rains; but for the most part Western Australia is a desert with an annual rainfall of less than ten inches.

Nevertheless, Western Australia is in some respects the most interesting region to the botanist in Australia, as it is here that the autochthonous Australian flora is seen at its best. Nearly four thousand species have been described, and of these a large majority are confined to Western Australia and include many of the most beautiful and peculiar of the Australian plants.

Diels[2] has written a very complete account of the flora of Western Australia, and in the introduction to this has given an excellent description of the most important botanical regions of Australia, with the more characteristic plants of each region.

West Australia is very old geologically, and it is believed that it was here that most of the peculiar Australian types originated. There is evidence that it was formerly separated from the eastern part of the continent, and from it, after the union of the two regions, the autochthonous plants migrated east and north.

Traveling westward by the transcontinental railway, which runs from Port Augusta, in South Australia, to Kalgoorlie, one obtains a good idea of the character of the country comprising much of the dry interior regions of Australia. The country was quite different in appearance from what I had anticipated. I had pictured the "desert" of the interior as a sandy waste, quite destitute of vegetation; but no such areas were seen, although they do occur in many parts of the central plains of Australia.

Along the route of the trancontinental there is, for the most part, no lack of small trees and shrubs. One region known as the "Nullarbor Plains" has only low bushes of salt bush (Kochia, Atriplex) and similar shrubs, much like the sage brush of the Nevada desert; but except in this region there were many trees, sometimes almost abundant enough to be called a forest; and among these were many shrubs of varying size, with bunch grasses and various low-growing plants between.

As usual, the predominant trees were Eucalyptus, and there were also shrubby species, known locally as "Mallee" (*E. oleosa*, *E. uncinata*). Casuarinas were also abundant, and Acacias were perhaps the commonest of the shrubs. As the latter were in full bloom, they made brilliant masses of gold in the dull gray-greens of the general vegetation. Other characteristic shrubs of this region are sandalwood (*Santalum cygnorum*), *Myoporum* sp., and "Quandong" (*Fusanus acuminatus*).

In many places, grass was sufficiently abundant to indicate excellent grazing country, if only artesian water were available, as the country is quite destitute of any natural streams or springs.

Aside from the Acacias and a few other shrubs, not many flowers were

[2] Die Vegetation der Erde 7. Leipzig, 1906.

seen. At one place, where the train halted, were some pretty everlastings, and some of the passengers brought back bunches of the gorgeous scarlet "Sturt pea" (*Clianthus Dampieri*), one of the most magnificent of Australian flowers.

The transcontinental terminates at Kalgoorlie, where a tiresome day was spent waiting for the train which was to take us on to Perth. The one-time famous mining centre was now almost dead, and the dreary country about offered very little to tempt the botanist, as the barren, sandy waste outside the town showed only scattered clumps of dwarf Eucalyptus scrub, and a few not particularly interesting flowers.

The train for Perth left in the evening, and the next morning we saw something of the wonderful floral display for which Western Australia is famous. All the way to Perth the railway ran through a veritable garden of flowers, including some of the most interesting and beautiful of the West Australian species. Cycads (*Macrozamia Fraseri*) and big grass trees (*Xanthorrhoea* sp.) were very abundant in many places, and here and there the ground was carpeted with solid masses of beautiful pink and white everlastings. Most beautiful of all were clumps of *Leschenaultia formosa*, a member of the Goodeniacae, of a blue so pure and intense as to excel anything I have ever seen—a sight never to be forgotten.

Bright yellow Hibbertias, blue Dampiera, scarlet, yellow, and purple Papilionaceae, pink Boronias, and many others which could not be identified mingled in this gorgeous show, and for the first time I saw one of the most extraordinary of the West Australian flowers, the so-called "Kangaroo paws" (Anigozanthos)—red, yellow, scarlet and green, or pure green. It was a most promising introduction to the floral wonders of this favored region.

Perth, the principal town of the western state, is an attractive city of moderate size, which is an excellent place to see the flora of the coastal region. While there is no formal botanical garden, the city, with great wisdom, has reserved as a park, and left practically untouched, a tract of considerable size along the water front, in which the plants are protected and where one can see most of the beautiful flowers which abound in this region.

The park extends along the bluffs of the river bank, and is an open forest in which the most important tree is the red gum (*Eucalyptus calophylla*), a very handsome and distinct species. An avenue of the splendid scarlet gum (*E. ficifolia*) has been planted, but I was too early to see it in flower. Many Casuarinas of fair size grow among the gums, and two Banksias, one of which, *B. grandis*, was a most striking object. The coarsely serrate leaves are said to be the largest leaves borne by any West Australian tree, and the immense cylindrical yellow inflorescences are extremely conspicuous.

The gritty, sandy soil was covered with a mass of varied vegetation.

Mingled with coarse grasses and sedges was a bewildering variety of low flowering shrubs and bushes. Many of these genera had also eastern species —*e.g.*, Boronia, Dampiera, Hibbertia, Patersonia, Tetratheca, and others; but many belonged to genera mainly developed in Western Australia and quite different from any seen before. The genus Candollea (Stylidium), while occurring in Eastern Australia, is particularly developed in Western Australia, where it includes a great many species, some dainty little plants a few inches high, with delicate pink and white flowers, forming dense patches; others taller and more robust, solitary or few together. The column formed of the stamens and pistil is bent backward and when touched springs out with a jerk, but gradually returns to its original position. They are popularly known as "trigger plants."

The genus Drosera is extraordinarily developed in Western Australia. Some of these species form tiny rosettes, close to the ground, while others have slender half-climbing stems four or five feet long, with flowers almost an inch in diameter.

Small everlastings (Helichrysum?) made solid patches of white in the sandy soil, and another very conspicuous plant was *Ricinocarpus* sp., one of the Euphorbiaceae—covered with masses of snow-white tubular flowers.

The pretty blue and lavender Patersonias were common, the sole representatives of the Iris family; but the Liliaceae were abundant, comprising some very pretty species of Dianella, Thysonotis, and Burchardia. The genus Thysonotis, of which there are a number of species in West Australia, has beautiful fringed petals, usually blue or purple in color. Burchardia has umbels of white flowers, not unlike some species of Allium, or the Californian Brodiaea.

A conspicuous and abundant plant was *Anigozanthos Manglesii* or "Kangaroo paws," a most extraordinary and bizarre flower. The scapes, two or three feet high, have closely set, two-ranked flowers. The tubular flowers are split open and flattened, so that the flower presents a fancied resemblance to the paw of an animal, the six short perianth segments representing the toes. As the outer surface of the perianth has a velvety surface, the comparison is still more striking; but the color is perhaps the most remarkable feature of this species, the perianth tube being of an intense verdigris green, while the inferior ovary is blood-red.

Ground orchids are very common and comprise some very beautiful species. As elsewhere in Australia, the genus Caladenia is the commonest. A fine yellow species (*C. Falda*) was particularly abundant, and when growing in quantity suggested beds of yellow Erythronium. Another species, *C. Patersonii*, is known locally as "spider orchid," as the sepals are drawn out into long, slender filaments. A third species, *C. gemmata*, was a deep blue, and extremely handsome. Other genera, Thelymitra, Glossodia, and Diuris, were represented by several species. Some of the Thelymitras, with racemes of fine azure-blue flowers, are among the handsomest of the orchids.

Grass trees (*Xanthorrhoea* sp.) were abundant, as in other parts of Australia, and Leptospermum, Melaleuca, and the peculiarly West Australian Verticordia, were the most common Myrtaceae aside from Eucalyptus. Some of the species of Verticordia, with delicate pink, finely fringed petals, were particularly noteworthy.

The only gymnosperm was a very abundant cycad—"Zamia palm" in the vernacular (*Macrozamia Fraseri*). This cycad is often responsible for the poisoning of animals which eat the young foliage, especially in dry seasons, or after a fire.

The hills about Perth offer many attractions to the botanist, as they abound in the beautiful flowers for which the whole region is famed. In September and October, the Australian spring, the magnificent Western Australian flora may be seen here in all its glory.

Western Australia is the home of many species of Eucalyptus, including the important timber trees jarrah (*E. marginata*) and karri (*E. diversicolor*). The former has a pretty wide range, usually growing in poor soil. It has a very characteristic habit, the stiff, ascending branches presenting a very different appearance from that of most of the gums. The karri is confined to a much more restricted area in the southwestern corner of the state, in a region of abundant rainfall and good soil. It is the giant among the western gums, and is said to attain a height of three hundred feet. It was a pitiful sight to see the gaunt skeletons of these splendid trees killed by ring-barking, to provide wretched grazing for a few sheep and cattle.

The most beautiful of the showy-flowered gums belong to Western Australia, most of the species having a very limited range. The best known of these, the splendid scarlet-flowered gum (*E. ficifolia*), is known only from a single locality about seventy miles from Albany. Another striking species is *E. macrocarpa*, a shrub of moderate size, whose stems and broad leaves are thickly covered with a white bloom, and whose solitary flowers, as big as a hollyhock, form big pompons of scarlet stamens. This species has broad, horizontal leaves like the juvenile foliage of the common blue gum, probably a permanent retention of the primitive leaf form.

A most interesting visit was made to Albany, on the South Coast. I had the good fortune to have as my traveling companion and guide Mr. C. E. Lane-Poole, Conservator of Forests. I am under great obligations to Mr. Lane-Poole, as well as other government officers, to whom I am indebted for many courtesies which greatly facilitated my work. Professor Sir Edgeworth David of the University of Sydney joined us, and the two days spent in the Albany district were among the most delightful and profitable of my Australian experiences.

While many species noted about Perth were found here, a great many were peculiar to the Albany district. The number of species within a small area was amazing, and it is probable that nowhere in the world could a greater number of species be found within an equal area.

The first day I was driven over a large area in the neighborhood of Albany, and the number of species met with was astonishing. At every stop new things were found, until one was fairly bewildered at the number of novelties. This was largely due to the remarkable number of species in such genera as Drosera, Stylidium, Hibbertia, and the innumerable Papilionaceae.

The region which provided this remarkable variety of interesting and beautiful plants was largely a sandy, moist, peaty moorland. This moorland was quite bare of trees or large shrubs in some places, but often there were groves of Eucalyptus, Casuarina, Banksia, and other small trees, as well as a dense scrub of species of Leptospermum, Melaleuca, Leucopogon, and various Leguminosae and Proteaceae. One of the most conspicuous of the latter was a Banksia, with brilliant scarlet flowers. Another remarkably showy shrub was a Callistemon, with huge scarlet bottle-brush inflorescences.

Among the hundreds of showy flowers it would be hard to decide which were the most characteristic. The Droseras were very abundant and of many species. Of the forty-five species found in Western Australia, probably the greater number occur in the Albany district. I failed to find the peculiar Australian pitcher plant (Cephalotus), which is known only from this region. The species of Stylidium were very numerous and varied, and one may guess that a large part of the 84 West Australian species are found near Albany. Various species of Goodenia, Dampiera, and Leschenaultia represented the characteristic Australian family Goodeniacae, and most of the genera already referred to as occurring near Perth were abundant about Albany, but generally represented by different species. Thus the green and scarlet "Kangaroo paws" of the Perth region was replaced by red and yellow, or yellow and green species. Boronia and Tetratheca are especially abundant in West Australia and include some very attractive species.

As elsewhere in Australia, Leguminosae are very much in evidence. Acacias in great variety abound, and are usually known as "wattle." The Papilionaceae are everywhere extremely abundant and comprise a great number of showy species. The colors are often extremely brilliant and the flowers are produced in great profusion. Many genera—Brachyzema, Chorizema, Gastrolobium, Jacksonia—are either entirely West Australian or predominantly so.

The Umbelliferae are fairly well represented and include some very peculiar types. Perhaps the most striking genus is Actinotus, one species of which, *A. Helianthi* of New South Wales, is known as "flannel flower," the inflorescence being very suggestive of the Swiss Edelweiss. In Western Australia the showy "southern cross," *A. rotundifolia*, is common. Space forbids a further enumeration of the Albany flora, which I think is the most varied that I have ever seen.

In company with Mr. Lane-Poole and Professor David, I visited Denmark, some forty miles from Albany, and once an important lumbering

district; but now most of the fine karri timber has been cut or killed, and only a few remnants of the great forest are left in this district. This region has a rainfall of approximately 60 inches, and the Western Australian forest reached its maximum development here.

Our means of transport was a motor railway "trolley," a small open car that was just sufficient to hold the three of us in addition to the driver. It was a decidedly novel experience and rather alarming at first, but we soon grew used to the motion and could enjoy the many interesting plants growing along the route. The track was often bordered with shrubs covered with beautiful flowers of all colors, and in many places were veritable forests of grass trees in full flower. In addition to the Xanthorrhoeas, there were also many specimens of the peculiarly West Australian Kingia, much like Xanthorrhoea in habit, but having small globular inflorescences on short stalks, looking like drum-sticks. The grass trees reach their greatest development in this region. They often branch, and the tall flower spikes, eight or ten feet high, stand out conspicuously above the crown of slender, drooping leaves.

The scenery in the vicinity of the karri forests is attractive. Owing to the abundant rainfall, there are clear streams of considerable size and rich and varied vegetation. Near the river banks grow the largest of the Banksias, *B. verticillata*, a tree 60 or 70 feet in height, with a trunk two feet thick.

The abundance of Loranthaceae throughout Australia has already been mentioned, but the most extraordinary member of the family is confined to Western Australia. This is *Nuytsia floribunda*, known in some districts as "Christmas tree" as it flowers at Christmas time; from all accounts it must be a most magificent sight. I saw many individuals, but none in flower. It is a small tree, a root parasite, and when in flower is said to be completely covered with a mass of orange-red flowers.

Western Australia is peculiarly interesting to the botanist, as the flora is almost exclusively made up of the strictly Australian types. The Malayan elements, which are so conspicuous in the forests of Queensland and New South Wales, are almost completely absent from Western Australia. The flora is also notably poor in ferns and bryophytes, few of which find a congenial home in this region with its poor, sandy soils and long, hot, dry summers.

Fortunately for the botanist, the region about Albany is not well adapted to agriculture and is likely to remain for a long time a happy hunting ground for the flower lover.

The autochthonous Australian flora reaches its extreme development in Western Australia, and there is an almost complete absence of any forms related to either the Malayan flora of Queensland and New South Wales, or the Fuegian element developed in Tasmania and the higher mountains of Eastern Australia.

A very large majority of the 4,000 species of West Australia are confined to that state, and there are many endemic genera as well as species. A notable feature of the flora is the remarkable number of species within a genus.[3] Drosera has 45 species, Candollea 84, Boronia 44, Goodenia 48, Grevillea 70.

Wallace (Island Life, 2d edition, p. 494) believes that Southwest Australia represents the remnant of an ancient, more extensive, isolated land area within which were developed the ancestors of the present autochthonous Australian flora, and this view seems to have much probability. There is strong evidence, as has already been stated, that in Cretaceous times Western Australia was completely separated from the eastern part of the continent, the western portion of which was probably united with New Guinea. The flora of North Queensland and coastal New South Wales still retains a large Malayan element, the "scrubs" of Queensland being predominantly Malayan in their flora.

It is highly probable that in early Tertiary times, before the Union of East and West Australia, the former region was occupied by a flora of exclusively Malayan character, while in the western continent the ancestors of the modern Myrtiaceae, Proteaceae, and other characteristic Australian types were completely segregated.

It is not unlikely that this western continent, owing to the intrusion of the sea in Cretaceous times, had a more uniform and less arid climate than now prevails in most of West Australia. It may have been like that now found in the extreme southwest, where a great majority of the existing species occur.

With the establishment of the connection between east and west, the existing climatic conditions were developed within the great arid central regions which occupy so much of the present continent.

With the increasing aridity in Western Australia is to be associated the evolution of the predominant xerophytic habit found in most of the typical Australian plants. These xerophytic forms apparently migrated east and north and took possession of most of the eastern territory. At the present time, all that is left of the ancient flora of northeastern Australia is probably the "scrub," confined to the narrow coastal belt of Queensland and New South Wales. This scrub is not continuous, but is surrounded by much larger areas of xerophytic Eucalyptus forest.

A few species of Eucalyptus and the allied genera, Tristania and Angophora, grow in some of the rain forests, and it may be that the few Proteaceae which occur in the rain forests, like *Grevillea robusta*, Macadamia, and Stenocarpus, are descendants of Western immigrants which have become adapted to the changed environment.

If the assumption is correct that the autochthonous Australian vegetation originated in Western Australia, the question then arises as to the origin of the ancestral forms from which this flora descended.

[3] Maiden, *loc. cit.*, pp. 183–199.

The region which shows most similarity to West Australia in its flora is the Cape region of South Africa. There is a similar development of Proteaceae, as well as certain Compositae common to the two, and the true heaths (Ericaceae) of the Cape are very similar in many respects to the Australian Epacridaceae. There are, however, many differences, and any land connections that may have existed must have disappeared at a very remote period. There is clear evidence of a connection of all the southern land masses in the Permian—the so-called "Gondwana Land," but the evidences for later connections are, at present, more or less problematical. However, it seems pretty certain that some connection between South Africa and Australia did exist, perhaps in the Tertiary, and that there is a real relation, although remote, between their floras.

It may be that further investigation will show that in the Tertiary, as was the case in the northern hemisphere, there was a practically uniform flora, occupying a more or less continuous land mass connecting the now widely separated regions of South America, Australia, and South Africa. It is possible that the common elements in the floras of Australia and South Africa are descendants of this ancient flora, which through long isolation have diverged from each other.

The complete isolation of Western Australia has resulted in a remarkable degree of specialization among a relatively small number of original types, with almost no admixture of immigrants subsequent to the severing of the connection of Australia with the land to the south.

EXPLANATION OF PLATES

Plate XXI

Fig. 1. Tree ferns, Botanical Gardens, Melbourne.

Fig. 2. *Banksia grandis*, Perth.

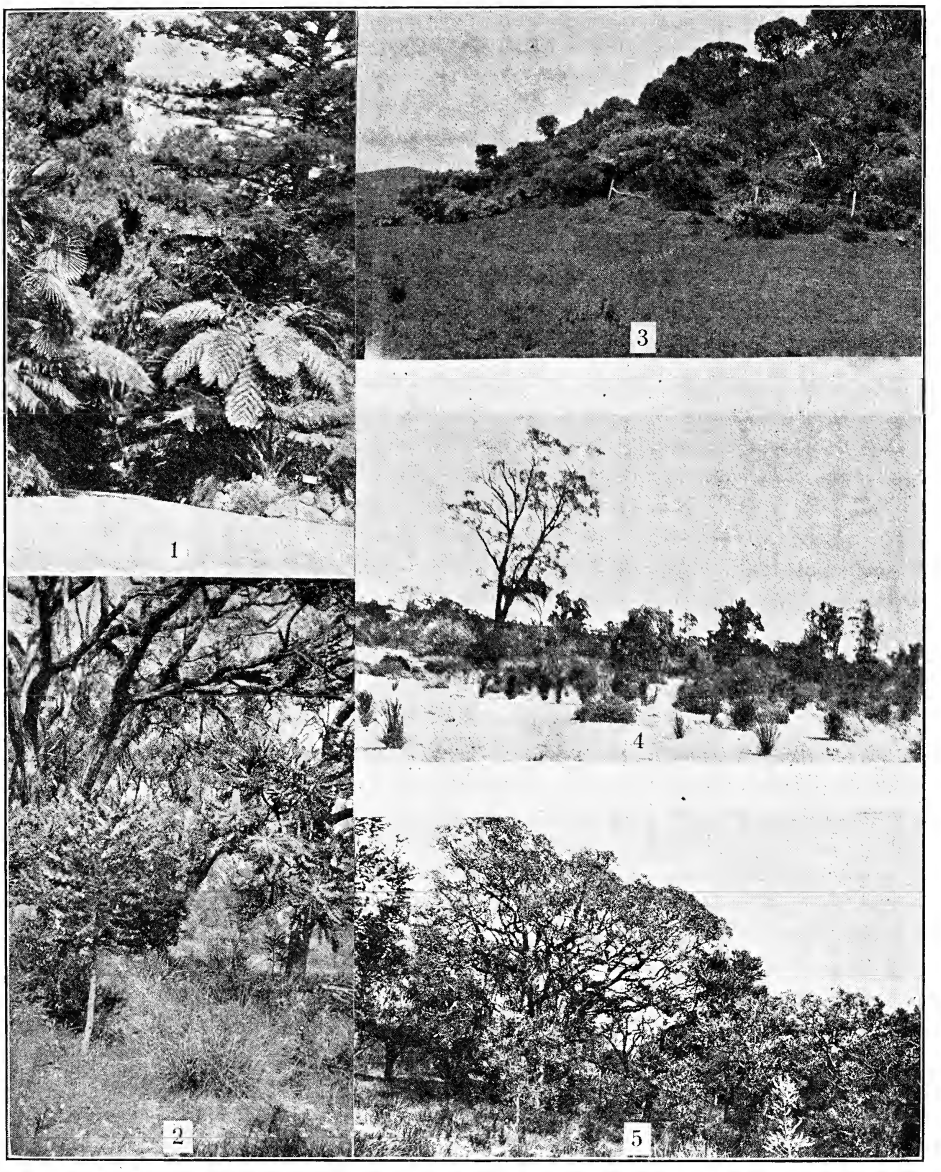
Fig. 3. Chaparral formation, National Park, Victoria. The trees are *Casuarina* sp.

Fig. 4. Desert vegetation, transcontinental railway.

Fig. 5. Coastal vegetation, Perth. The largest tree is a red gum (*Eucalyptus calophylla*).

Plate XXII

West Australian grass trees (*Xanthorrhoea reflexa*). Photograph furnished by Mr. C. E. Lane-Poole.



CAMPBELL: AUSTRALASIAN BOTANICAL NOTES

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EVOLUTION AND GEOGRAPHICAL DISTRIBUTION OF THE GENUS VERNONIA IN NORTH AMERICA

HENRY ALLAN GLEASON

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The genus Vernonia, with its vast assemblage of over 500 species, ranges through the western hemisphere from Argentina to Manitoba, occupying a region of great climatic variation and habitats of great ecological diversity. In preparing manuscript for the treatment of the genus in the "North American Flora," 123 species have been recognized north of Colombia and Trinidad. Within this number a few stand comparatively isolated from all the others, while many are so closely related in form and structure and so similar in distribution that they must be closely akin genetically. Over 30 species-groups may be distinguished in this way. Within these minor groups evidences of specific evolution correlated with geographic distribution are frequently seen, while most of the groups, considered each as a whole, present strong evidence in favor of their relation to, and probable origin from, each other. It is therefore possible to build up a general scheme of evolution and migration within the genus in which the two lines of evidence, structural and geographic, complement and support each other.

There can be little doubt that the ancestral home of the genus, as far as North American species are concerned, is tropical South America. This is shown by the presence there of a large number of species of greater structural diversity than exist on the North American continent, and also by the fact that many South American species are of a structure which clearly indicates their primitive nature.

Within the genus as a whole, the more fundamental structural differences, which have been used in the division of Vernonia into its many sections and subsections, relate chiefly to the structure of the achenes, the pappus, and the involucral scales. Nothing can now be said concerning the possible evolution of these groups. Of those distinguished by Bentham and Hooker in "Genera Plantarum" and accepted by Hofman in "Die natürlichen Pflanzenfamilien," four have reached North America.

1. The section Stengelia, of the East Indies, with veiny, foliaceous involucral scales, is represented by a single species, *V. anthelmintica* (L.) Willd., sparingly introduced into a few islands of the West Indies. Certain Mexican species which bear a superficial resemblance to this section appear to belong rather to the section Lepidaploa, and their similarity to Stengelia is better explained by convergent evolution.

2. The section Tephrodes, of the paleotropical region, with terete achenes, is represented by a single species, *V. cinerea* (L.) Less., widely introduced

as a weed in tropical America and recently reported from extreme southern Florida. Its further migration through the agencies of commerce is to be expected.

3. The section Stenocephalum, with coriaceous, spine-tipped involucral scales, represented by several species of tropical South America, has a single little-known and recently discovered species, *V. jucunda* Gleason, in the mountains of southern Mexico. If we assume from the negative evidence at hand that the section is actually absent from Central America, we may infer an early migration of the section northward, followed by extinction in Central America and the isolation of the single species in Chiapas.

4. The section Lepidaploa, with membranous involucral scales and ribbed achenes, includes 120 species of North America and many more in South America.

Within this large section there is still well-marked evidence of evolutionary development in structure, illustrated most plainly by the character of the inflorescence. One type of inflorescence may be assumed *a priori* as the most primitive, and from it by successive small changes all the other types may be derived. Since the center of evolution and migration for the genus is considered to be tropical South America, where this primitive type is largely developed, and since the succeeding stages in the modification of the inflorescence occur progressively farther to the north, structure and distribution complement each other, and it may be assumed with little hesitation that migration and structural evolution have proceeded simultaneously; that the tropical species, while not necessarily the oldest in time, are at least the most primitive in structure, and that the outlying species of the temperate part of North America are both young in age and advanced in evolution. It can not now be stated whether there is a similar correlation between structure and distribution among the species of South America.

In the section Lepidaploa, the inflorescence is either a scorpioid cyme or some other type of cluster obviously derived from it by certain apparent modifications in the original structure. In such an inflorescence each head is morphologically terminal; a lateral branch, arising at the first node below the involucre, terminates in a second head and bears another lateral branch which behaves in the same way. There is thus produced a more or less elongate sympodial axis, morphologically indeterminate in its development, and with its series of truly terminal heads apparently lateral and secund along it. Since the successive lateral branches arise from nodes, which are normally marked by leaves, it may be assumed at once that the leafy scorpioid cyme is the primitive inflorescence, while those species in which the bracteal leaves are suppressed stand relatively higher in the scale of evolution.

Each segment of such an inflorescence then consists of a basal internode with a leaf at its summit and a head beyond it. The structure of the bracteal leaves varies greatly, but in general they maintain the form and pu-

bescence of the cauline leaves and differ from them chiefly in size. The head is pediceled if separated from the bract by an obvious internode, and the inflorescence is then a scorpioid raceme. If this internode is reduced, the head is sessile and the cluster becomes a scorpioid spike. The clusters may be straight or flexuous, long or short, crowded or loose, with heads ranging from 2 to 25 in number. As a result of the straightening of the sympodial axis, the heads appear lateral and are usually placed about 90° around the axis from the bracteal leaf.

The leafy scorpioid cyme is found in 57 species, ranging throughout the West Indies and on the continent extending north into southern Mexico. Species with leafless cymes, 63 in number, occur commonly on the continent from Panama to New England. From this region four have crossed the narrow gulf east of Yucatan and entered Cuba; one has reached the Bahamas from Florida; one is endemic to St. Vincent, and another reaches Trinidad and the neighboring islands. In general, the distribution of the latter group is continental, of the former Antillean.

Perhaps the simplest type of the primitive leafy inflorescence is found in the species-group Graciles, in which the cymes are stemlike and quite undifferentiated from the truly vegetative portion of the stem, with bracteal leaves closely resembling the cauline in size and shape. Species of this group are almost entirely South American, ranging, according to Ekman, from Colombia to eastern Brazil. One species only, *V. gracilis* H. B. K. var. *tomentosa* Ekman, of Bequia, occurs in our range.

A second group, with almost equally simple inflorescence, composed of long, irregular, branching cymes, with long internodes and leaflike bracts, is the Argyropappae, of tropical South America, Central America, and southern Mexico. The South American origin of the group may be assumed. From there, *V. remotiflora* Rich. has been introduced into St. Thomas; *V. acilepis* Benth. is endemic to Costa Rica, and *V. argyropappa* Buek extends from Peru to Mexico. Two offshoots of the latter have arisen in Mexico, *V. hirsutivena* Gleason in Yucatan and *V. ctenophora* Gleason in Campeche, differing in minor structural details.

It will be observed that these two groups, simplest in structure, are distributed primarily in South America and that only a part of their species reach North America, although among these are three endemic species and one endemic variety.

A third group of similar primitive structure as to inflorescence is the Schiedeanae, of Central America and southern Mexico. While its members differ sharply from the preceding group in their large heads, the peculiar development and specialization of the involucral scales, and the absence of foliar resin dots, they retain the simple cymes and broad, heavy leaves, and may possibly be derived from it. *V. vernicosa* Klatt, with narrow acuminate scales, appears to be the simplest and is endemic to Costa Rica. *V. Seemanniana* Steetz follows in Costa Rica, with broad, obtuse scales,

and the greatest modification is found in *V. Schiedeana* Less., ranging from Honduras to Vera Cruz, with involucral scales broadly dilated at the tip. The progressive specialization in structure, correlated with increasing distance to the north, is here clearly shown.

There now follow seven species-groups with 33 species, all West Indian, all clearly related, and all exhibiting a remarkable correlation between structure and distribution.

The most primitive of these, from which the other six are directly or indirectly derived, is the Arborescentes, ranging from the Windward Islands to Jamaica. The wide range and primitive structure probably indicate an early arrival in the region. The most primitive species, *V. icosantha* DC., has stems bearing leaves of normal size to the apex and terminating in a single sessile head. At the base of this head the two primary cymes arise; they are straight, elongate, with prominent internodes, sparingly branched or simple, and bear numerous heads. The chief distinction in the inflorescence between it and the Graciles is the regular presence of paired primary cymes. In *V. sericea* L.C.Rich., of the Virgin Islands and Porto Rico, the cymes are shorter and more frequently branched. *V. borinquensis* Urban, of Porto Rico, has exceedingly flexuous, many-headed, freely branched cymes, the branches invariably arising at the base of a head. *V. arborescens* (L.) Sw., of Jamaica, has numerous frequently congested cymes and reduced bracteal leaves. *V. permollis* Gleason, of Jamaica, completes the group, with congested cymes and an unusual development of foliar pubescence. The general tendency of the group is toward the production of cyme-branches and supernumerary cymes, making a congested inflorescence in which the bracteal leaves are reduced.

The Longifoliae, a group of three species, is related through *V. longifolia* Pers., of the Lesser Antilles, to *V. icosantha*. Superficially the two species are much alike, but the inflorescence in the former shows a distinct difference. The primary cymes are short, compact, divergently spreading at an angle of 60–90°, crowded, bearing only 2–5 heads on short internodes with bracteal leaves considerably smaller than the heads. Secondary cymes arise just below the primary in the upper leaf axils. They are essentially leafless for the first 2–5 cm., and then bear toward the summit either the usual crowded heads or a terminal head subtended by two short cymes. This whole inflorescence terminates completely the growth of this portion of the stem, but during the next vegetative season new branches appear from the next lower axils in order, grow out at a divergent angle, soon surpass the old cymes of the previous season, and at the next blooming season bear their cymes in turn. The plant has therefore a method of continuing its vegetative growth beyond one season, and as a result reaches a considerably larger size. *V. Shaferi* Gleason, of Montserrat, is closely similar, and represents an island endemic. *V. albicaulis* Pers., of the Virgin Islands and Porto Rico, preserves the same inflorescence but differs in its obtuse or broadly rounded leaves.

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hydrogen-ion concentration of the iron-free nutrient solution at pH 4.2, the growth of young wheat plants shows ferric citrate to be decidedly the most available form of iron. In this form, 10 milligrams of iron per liter appear to be sufficient for the growth of these plants.

With the exception of the less favorable results from $FeSO_4$ as compared with its high solubility, the variations of efficiency in these various forms of iron can be attributed either to differences in solubility or to different modifying effects upon the pH value of the nutrient solution. Thus, ferric citrate reduces the hydrogen-ion concentration and acts favorably, while $Fe_2(SO_4)_3$ increases the concentration of this ion and is an injurious source of iron. The inferiority of $FeSO_4$ as compared with ferric citrate may reside in toxic properties of the former, as suggested by Jones and Shive (9).

It seems probable that the formation of either $Fe(OH)_3$ or basic iron salts by hydrolysis of inorganic salts of iron will render these unavailable in general as sources of iron in nutrient solutions. Yet ferric hydroxide may be superior to ferric phosphate as a source of iron in some cases, as indicated by a previous observation (13) that barley seemed to acquire a much improved supply of iron from a nutrient solution containing suspended ferric phosphate when ferric hydroxide was also added. Apparently the use of $FePO_4$ should be discriminative, because of its low solubility. These results confirm previous observations of the favorable effects of iron applied to nutrient solutions in the form of salts of organic acids. The conclusions find support in both the appearance of plants, especially as to intensity of green color of the leaf, and the data of plant measurements.

Summary

1. Results are here given relative to the solubility and availability to young wheat plants of various compounds of iron in a particular form of nutrient solution.

2. Solubility tests at different pH values of the nutrient solution have shown that ferric phosphate is relatively insoluble. This is true of ferric and ferrous sulphate at a hydrogen-ion concentration of the iron-free nutrient solution approaching neutrality. While ferric citrate is not very soluble, it possesses the advantage of remaining soluble over a considerable range of pH values of the nutrient solution.

3. Ferric sulphate increases the hydrogen-ion concentration of the nutrient solution here used, while ferric citrate causes the opposite effect. The other forms of iron tested have little influence in this respect.

4. The growth measurements of the young wheat plants show that ferric citrate was decidedly the most favorable form of iron here employed. The variation in efficiency of iron in the forms supplied is correlated with variation either in the solubility of this element or in the modification of the pH value of the nutrient solution. The results show clearly that ferric phosphate is likely to be inefficient because of its low solubility.

5. Ferric citrate supplied at the rate of 10 milligrams of iron per liter of the nutrient solution employed here is not completely dissolved, but seems to provide abundant iron for the growth of the young wheat plant, where the nutrient solution is renewed.

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THE EFFECT OF THE HYDROGEN ION ON THE PROTOPLASM OF THE ROOT HAIRS OF WHEAT

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In modern experiments with nutrient solutions in which phosphates are provided as either potassium mono-hydrogen or potassium di-hydrogen phosphate, it has been found by several writers that in fairly high concentrations these phosphates are injurious. Hoagland ('17), Duggar ('20), and Salter and McIlvaine ('20), indeed, found that if the concentration of phosphate was increased to but a slight extent, its injurious effect was marked. Since phosphorus is essential for the growth of plants, and is actually a constituent of protoplasm, it is not at once clear why its influence should so suddenly change from beneficial to harmful as its concentration is increased in the culture solution. The unquestioned nutritive value of the phosphate ion and of the potassium ion directs attention to the other component of the salt, the hydrogen ion. A logical first step in determining the nature of the harmful effect might be to ascertain by inspection whether it is general or local; to see if any particular cells behave or develop differently in solutions containing a large amount of phosphate than in solutions in which the concentration is lower.

For this purpose seedlings of spring wheat (*Triticum vulgare*), "Marquis" strain, were grown in solutions selected from Shive's ('15) "optimal" group. The total osmotic pressure of each of these solutions was calculated by Shive to be about 1.75 atmospheres. Since the lime-magnesium ratio has been thought to influence the growth of plants,[1] solutions were chosen in which this ratio was practically constant. The compositions and hydrogen-ion concentrations of the solutions employed are shown in table 1.

The hydrogen-ion concentrations were determined colorimetrically with a colorimeter of the form described by Bock and Benedict ('18). The indicator used in these experiments was tetrabromphenolsulphonephthalein (brom-phenol blue), prepared according to the directions of Clark ('21). The buffer solutions used were mixtures of potassium hydrogen phthalate and hydrochloric acid, and of potassium hydrogen phthalate and sodium hydroxide (Clark, '21); they were standardized by titration with dibromthymolsulphonephthalein (brom-thymol blue) against standardized hydrochloric acid obtained from the LaMotte Chemical Products Company of Baltimore, Maryland.

Baker's "analyzed" chemicals were used for the culture solutions, which were made up from single-salt stock solutions drawn from covered

[1] For a general discussion see Lipman ('16).

burettes and diluted to the required volume with water that had been condensed in block tin and stored in a paraffin-lined jar.

TABLE 1. *Composition and Hydrogen-ion Concentration of Solutions Employed*

Solution Number	Shive's Designation	Hydrogen-ion Concentration pH[2]	Composition Partial Concentration in Grams per Liter of Solution		
			KH_2PO_4	$CaNO_3.4H_2O$	$MgSO_4.7H_2O$
1.....	R_2C_4	3.94	0.98028	2.45600	4.92984
2.....	R_4C_3	3.85	1.96056	1.84200	3.69738
3.....	R_5C_2	3.68	2.45070	1.22800	3.69738
4.....	R_6C_2	3.60	2.94084	1.22800	2.46492
5.....	R_8C_1	3.47	3.92112	0.61400	1.23246

3 drops of a 0.001 M solution of Fe_2Cl_6 were added to every 325 cc. of nutrient solution.

The seeds were germinated on moist, unglazed pottery, and were then placed on paraffined cotton netting stretched over the tops of glass tumblers of tap water, where they remained for three or four days, or until the seedlings were from four to six centimeters high. From these, plants were selected for uniformity in size, color, and development, and were transferred to culture bottles containing the nutrient solutions, which were changed at intervals of from three to five days. The culture bottles were of flint glass, with wide mouths and cork stoppers, and had a capacity of about 325 cc. Each was enclosed by two jackets, the inner one of heavy black paper, the outer one of heavy light-colored manila paper. Four holes were bored in each stopper, which was then immersed for a few minutes in hot paraffin, and one plant was fixed in each hole with a pledget of non-absorbent cotton. The cultures were kept in a greenhouse at a temperature of 20° to 25° C. throughout the winter and spring months.

Although differences in the length and general appearance of the plants were not noticeable during the first few days that the plants grew in the nutrient solutions, there were appreciable differences in the root systems before the third day. The roots of plants in solution 1 were long and straight with several long secondary roots; those in solutions 4 and 5 were short and stubby. Some of the roots in solution 5 were branched very near the tips; these branches were hardly more than tubercles. The secondary roots in solutions 4 and 5 were thick, much branched, and bore an unusually large number of root hairs, some of which were so long that their ends projected beyond the tip of the root itself. Plants in solutions 2 and 3 presented appearances intermediate between the two extremes; the roots were of medium length and were moderately branched, but they were not stubby. At the end of a week the differences between cultures were even more apparent. It thus appears that short, stubby, branched root systems are associ-

[2] These pH values are somewhat smaller than those obtained by McCall and Haag ('20) and by Meier and Halstead ('21). However, since it is the relative concentrations that are of value in the present experiments, the actual pH is unimportant.

It is a comparatively short distance across open water to the north of Porto Rico, the home of *V. albicaulis*, to the southern islands of the Bahamas. In these southernmost islands occurs *V. bahamensis* Griseb., the most primitive member of the species-group Bahamenses. The fundamental difference between this group and the Longifoliae is again found in the inflorescence. Here the cymes, after the flowering period, continue their elongation into the vegetative shoots of the next season. Not every cyme necessarily elongates, but there are regularly 2–4 such branches at the apex of each year's growth. Toward their base, paired scars mark the location of former heads and bracteal leaves, while above them scars in spiral arrangement indicate the former position of foliage leaves. All these Bahaman species are therefore bushy, widely spreading, freely branched shrubs. It is particularly worthy of note that they all have broad obtuse to retuse leaves; that *V. bahamensis*, the species most nearly resembling *V. albicaulis* in leaf form, is the species of the southernmost islands, and that the particular specimen in herbaria which most closely approximates the leaves of the Porto Rican plant in size was collected on South Caicos Island, almost the extreme southeastern island of the group. *V. arbuscula* Less. and *V. obcordata* Gleason occur farther to the northwest in the Bahamas. *V. complicata* Griseb., of eastern Cuba, differs only in minor characters. It is difficult to imagine a more striking instance of correlation between structure and distribution than is presented by this group in its relation to the Longifoliae.

The last three species-groups illustrate the following course of development in the inflorescence:

1. The cyme is a special branch with reduced bracteal leaves and elongate axis.

2. The cyme and leafy branches differ merely in position, and the inflorescence is compact.

3. The cyme becomes the leafy branch at the conclusion of the blooming season, and the inflorescence is compact and reduced.

The fourth species-group of the seven, the Racemosae, includes five species of Hispaniola and Cuba. They are probably derived from *V. sericea* of the Arborescentes, which is located near by in Porto Rico; *V. racemosa* Delp. was considered by Ekman a variety of *V. sericea*, and, like it, most of the species have leaves pubescent on the lower surface. In this group the two upper primary cymes are short, with only 2–5 heads. Below them, every leaf axil for a considerable distance down the stem produces similar short lateral cymes. The whole makes an elongate subcylindric inflorescence, quite different from the broad, spreading type of the preceding groups. Secondary vegetative branches apparently do not exist. Within the group, evolution is seen in the progressive reduction of the leaf surface, of the cymes, and of the number of flowers in the head. While *V. racemosa* of Hispaniola has lanceolate leaves, and cymes of 2–5 many-flowered heads,

the next three, one of Hispaniola and two of Cuba, have progressively narrower and more revolute leaves and smaller and fewer heads. The group culminates in a fifth species, *V. corallophila* Gleason of Cuba, with linear leaves revolute to the midvein, 11-flowered heads, and 1-headed cymes which appear as single axillary heads.

A fifth species-group, the Gnaphaliifoliae, also appears to be derived from *V. sericea* or some species similar to it. There is the usual terminal head, subtended by two primary cymes, and numerous other cymes arise from the upper axils. They are usually flexuous, spreading or ascending, and only occasionally branched. Such structures point unmistakably to an origin within the Arborescentes, with which they share many structural features and from which they are indeed rather weakly separated. The three species are all Cuban. In this group axillary branches do not continue the vegetative growth, but the whole herbaceous stem dies and is replaced by new growth from the perennial base.

All groups heretofore described in this general series have acute or acuminate involucral scales. The sixth, the Acuminatae of Jamaica, have obtuse scales, and also differ from the Arborescentes in their resinous-dotted, non-papillose leaves and in their flattened and twisted pappus bristles. Nevertheless, the simplest species of this group bears a general resemblance to *V. arborescens;* has been placed adjacent to that species by Ekman, and may have been derived from it. I fail completely to see any resemblance between this group and the Fruticosae, as has been claimed by Ekman. *V. acuminata* Less. is the common species of lower elevations. *V. pluvialis* Gleason, the high-mountain derivative, presents an inflorescence of short, much congested cymes, aggregated in subcapitate clusters.

The seventh and last group of this series, the Fruticosae, includes one species of Hispaniola and eight of Cuba, particularly of the mountains of the eastern part. Many of these are poorly known, some by a single collection, and the number of species may easily be subject to increase or decrease as further collections are accumulated for study. From the inflorescence standpoint, they exhibit the simplest scorpioid cymes to be found in the West Indies. They are mostly straggling vinelike plants with indeterminate growth. At some distance above the base the main axis ends in a terminal head, while immediately beneath it a lateral branch, diverging at a small angle, continues the sympodial axis and bears heads in the same way. The heads are separated by internodes about equal to those of the sterile section of the stem in length, they are subtended by bracteal leaves which in almost all species are virtually indistinguishable from the cauline in size and shape, and furthermore the cyme axis is frequently prolonged after flowering into a leafy, sterile stem. In their leaf habit and papillose pubescence they approach *V. gnaphaliifolia;* in other features they have no near relatives in the West Indies and apparently none in South America. Nevertheless, they offer no new structural features to separate them from

the preceding species-groups. In the lack of sufficient material, the evolution within the group can not now be discussed.

It is not necessary to presume that only one ancestral stock of Vernonia migrated into the West Indies. The seven species-groups just described, constituting probably one evolutionary stock, have spread over the whole region and developed into many species. Other stocks may also have immigrated from South America, been isolated in certain islands, and developed endemic species. Certainly two species-groups now exist whose relations can not be explained, and which should probably be considered as entirely distinct evolutionary lines. These are the Buxifoliae and the Sagraeanae.

The Buxifoliae include three species of the mountains of Hispaniola. They are characterized by glabrous achenes, heads in subcapitate clusters, and an unusually large number of involucral scales, arranged in a beautifully spiral imbrication.

The Sagraeanae include ten species, nine in Cuba and one in Hispaniola, with an outlying variety in Dominica, characterized by large glabrous achenes and usually by large many-flowered heads. Ekman would relate the group to the Bolivian *V. robusta* Rusby, which differs in achenes, hispid in the furrows, and in the number of setae of the pappus, about 25, instead of 40–70; also to the Bolivian *V. obtusata* Less. (*V. subacuminata* Hieron.) which has densely hirsute achenes. There is a superficial resemblance to these Bolivian plants in their heavy, rugose, reticulately veined leaves, and to *V. robusta* also in their large heads. On the ground that specialized involucral scales, few-flowered heads, and rigid, coriaceous, or tomentose leaves are characters which indicate an evolutionary advance, *V. Sagraeana* DC. and *V. viminalis* Gleason may be regarded as the most primitive species, and *V. Wrightii* Sch.-Bip. and *V. purpurata* Gleason as the most advanced.

We have now disposed of all leafy-bracted scorpioid species of North America except two, *V. yunquensis* Gleason and *V. segregata* Gleason. These Cuban species are poorly known and the former is represented in herbaria only by the type specimen. While each of them exhibits certain points of resemblance to other West Indian species, it is not possible to draw any conclusions as to their relationships.

The general affinities of the 57 species of the leafy-bracted groups may be summarized by the diagram (fig. 1), from which it may be seen that without exception the more advanced groups lie progressively farther from South America, that no group is common to the West Indies and the continent of North America, except as introduced, and that, with very few exceptions, the more advanced species of each group also lie farther away from the center of origin, either in horizontal or in altitudinal distance.

The 63 species in which the bracteal leaves are suppressed show certain fundamental differences among themselves in the structure of the inflorescence, as a result of which five well-marked evolutionary stages may be

distinguished. In the first of these the cymose structure is obvious, each cyme is more or less elongate with secund heads, and branches occur at such intervals that the scorpioid structure is not obscured. In the second, secondary branches are developed at the bases of a great many heads, so that three successive nodes without branches rarely occur. The result is a large branching cluster which bears little superficial resemblance to the

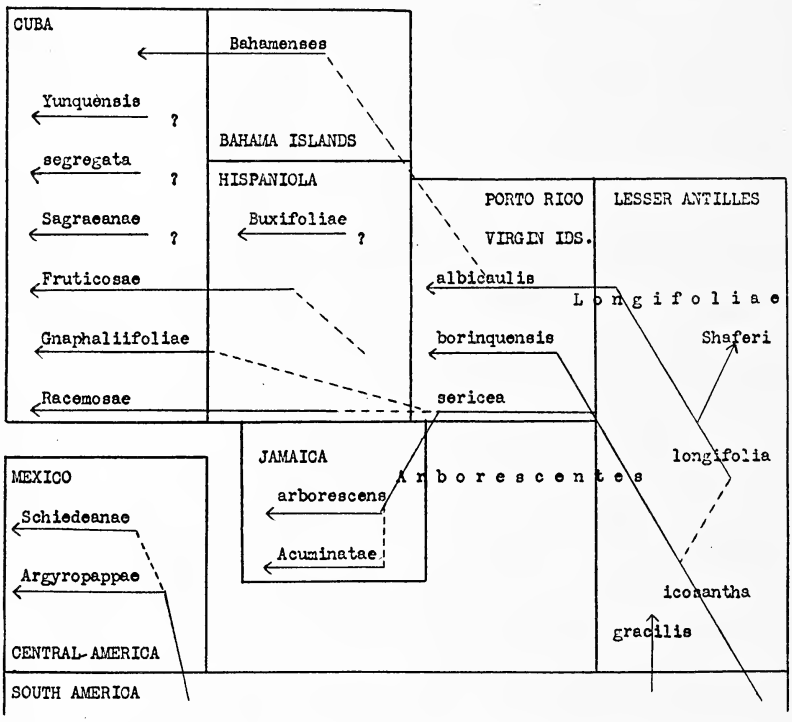

FIG. 1. Migration and evolution of the leafy-bracted Vernoniae of North America. Solid lines show distribution by their location, migration by the direction of the arrow. Dotted lines show probable connection by evolution between species-groups.

simple scorpioid cyme, although undoubtedly derived from it. In the third, the heads are suppressed at those nodes where secondary branches are developed. Since these appear at virtually every node, only terminal heads are produced on the cymes, and the whole cluster appears to be dichotomously branched. The fourth stage represents a much greater step forward. Here the basal internodes of the inflorescence are much shortened or almost suppressed, while the number of heads is greatly reduced. Since the terminal internodes retain a normal length, the whole inflorescence appears subumbellate. While in the first three stages new vegetative branches may arise from below the inflorescence, so that the stem may live several years and reach a large size, in the fourth type, as well as in the fifth, the appearance of the inflorescence prevents further growth

of the stem, and new vegetative parts appear only from the perennial base of the plant. In the fifth and last stage, only a few terminal and subterminal heads retain the umbellate arrangement, while from the upper stem axils similar clusters arise, producing a broad, flat-topped or hemispheric cluster with all the heads peduncled. These five stages are shown diagrammatically in figure 2.

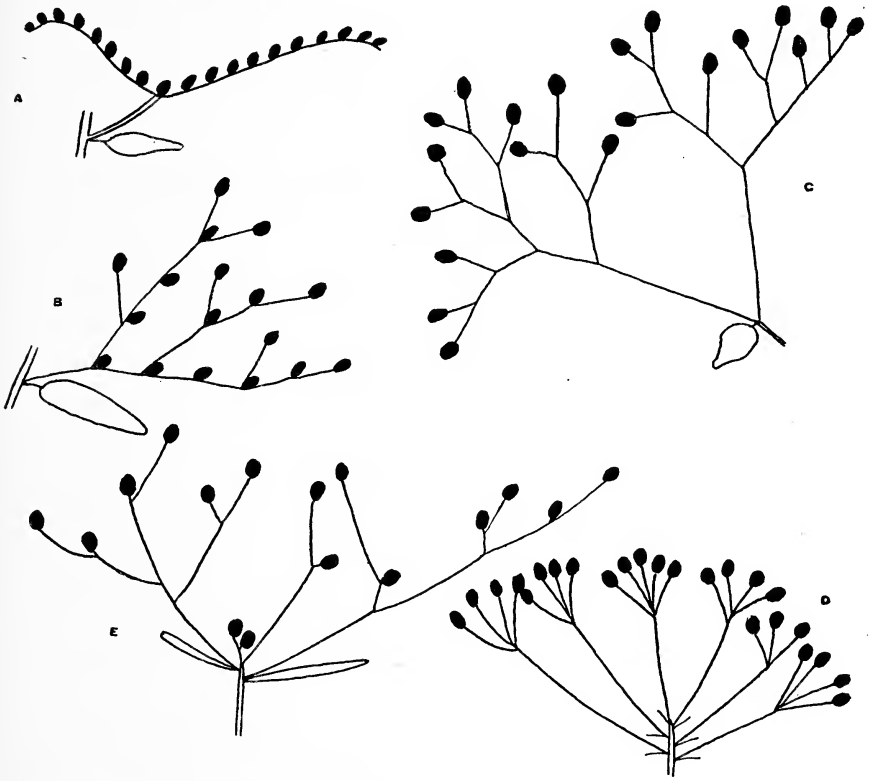

FIG. 2. Modifications of the inflorescence in the bractless Vernoniae of North America *A*. Stage 1, *Vernonia scorpioides*, lateral branch with two terminal cymes. *B*. Stage 2, *canescens*, lateral portion of the inflorescence. *C*. Stage 3, *havanensis*, portion of a terminal inflorescence. *D*. Stage 4, *Karvinskiana*, terminal inflorescence, with a few primary branches omitted. *E*. Stage 5, *texana*, complete terminal inflorescence. All figures diagrammatic as to position of branches or cymes, but accurate as to character of branching and proportion.

Other evidences of evolution appear within the last two stages, leading to the segregation of several species-groups.

The five stages are again well correlated with their geographical distribution. The first occurs in South America and is represented in our region by a single species of St. Vincent, *V. pallescens* Gleason, and by two which extend across the Isthmus of Panama into southern Central America, *V. scorpioides* (Lam.) Pers. and *V. brachiata* Benth. The second includes

several species of northwestern South America, some of which extend into Central America also, and several others ranging as far north as southern Mexico. Species of the third stage have crossed the narrow channel from Yucatan and are now limited to western Cuba; the fourth is confined to Mexico, and the fifth has four species in northern Mexico and 31 in the United States and the Bahamas.

The species-group Stellares, representing the second stage, includes the commonest species of the mountains of Colombia, Central America, and southern Mexico. The Colombian species *V. canescens* H.B.K. and *V. mollis* H.B.K. retain the primitive character of acuminate involucral scales; the former also extends north into Mexico, and the latter is doubtfully admitted into the North American flora. Of the remaining five with acute to rounded scales, *V. patens* H.B.K. occurs in both continents, while the others are strictly North American. The most advanced morphologically is *V. morelana* Gleason, which alone does not occur south of Mexico.

The third stage includes the species-group Menthaefoliae, undoubtedly closely related to the Stellares, but now isolated in western Cuba and the Isle of Pines, except for a few specimens from central and eastern Cuba as well, where they may have been recently introduced.

The fourth stage includes three well-marked species-groups, which are nevertheless closely related. The Umbelliformes include 9 species, mostly quite closely related and in some cases separated with difficulty. The simplest species (and the commonest in herbaria) have small heads, with short involucres and seldom more than 15 flowers; the more advanced have larger heads and taller involucres. One of these, *V. Conzattii* Robinson, with its abruptly rounded and mucronate involucral scales, marks a transition to the group Mexicanae, with three species in the higher mountains of southern Mexico. Here the scales are extraordinarily specialized, being 3–8 mm. wide, loosely spreading, at least at the tip, and prominently reticulately veined. The two closely related species of the Alamanianae have also large scales but lack the reticulate venation. The general evolutionary tendency of the series is apparently toward large heads and specialized scales, and this is correlated geographically with an ascent to higher levels in the mountains.

Passing now to the 35 species of the fifth stage of evolution, as shown by the inflorescence, we find the most primitive members in the Texanae, a group of four species, three in northern Mexico and one in Texas. Since the inflorescence has already passed to the paniculate stage, equally characteristic of the other species of the United States, evidence for the primitive character of the group must be sought in other characters. The leaves in all four species are more or less pitted beneath and the outer pappus bristles are poorly differentiated from the inner in width, both of which features occur also in the Umbelliformes. The most important primitive character, however, lies in the involucre, and has not been mentioned before because

it is shared by virtually all the groups hitherto discussed. Here the scales are relatively few in number and poorly imbricated. The inner scales are progressively more exposed than the middle and outer ones, contrasting plainly with the numerous regularly imbricated scales of most other species of the United States. Of the four species in the group, *V. texana* (A. Gray) Small, is best known and occurs in Texas, Louisiana, and Arkansas.

From the area of the Texanae, migration accompanied by specific evolution has proceeded in two directions, northward through the prairie region and eastward along the coastal plain. In each direction one or more of the primitive structures have been lost, until in Michigan and Massachusetts they have disappeared completely.

In northern Texas occurs the group Lindheimerianae of three species, two of which are suspected to be hybrids. *V. Lindheimeriana* Gray & Engelm., which is undoubtedly a good species, retains the primitive involucre and narrow outer bristles of the Texanae, from which it seems to be derived, and differs chiefly in its tomentose leaves and scales.

The Fasciculatae, extending from Texas and New Mexico northward and eastward to Manitoba and Ohio, retain the pitted leaves and present to a still greater degree the narrow, undifferentiated outer bristles of the pappus. They have lost the primitive involucre and have developed long heads with numerous scales imbricated with great regularity. It is noteworthy that the more southern species, as *V. marginata* (Torr.) Raf., still show a tendency toward acumination of the scales, as in Texanae, which character is lost to a large extent in *V. fasciculata* Michx., ranging from Nebraska to Ohio, and completely in *V. corymbosa* Schw., distributed along the Red River of the North in Minnesota, the Dakotas, and Manitoba, where it marks the extreme northern limit of the genus.

The peculiar local species, *V. Lettermanni* Engelm., of Arkansas and adjacent Oklahoma, bears a strong superficial resemblance to *V. fasciculata* and is possibly an evolutionary development from it. It retains the glabrous leaves with pitted lower surfaces and the congested heads with closely imbricated scales, like the latter species, but has broader, well differentiated outer pappus bristles.

The group Interiores takes its name from *V. interior* Small, which is undoubtedly the basic species. Common in central and northern Texas, where it overlaps the range of the ancestral Texanae, it extends north to Nebraska, and thence east to the Mississippi River. The involucre in this species has only partially lost its primitive structure; the outer pappus is narrow but nevertheless plainly differentiated; the leaves are broad, without pits, and characterized by multilocular hairs forming a more or less tomentose pubescence. *V. Baldwini* Torr. is an Ozarkian derivative, with broader outer pappus bristles, and with the acuminate involucral scales recurved at the tip and pubescent on the inner face. The species is probably of recent origin, and specimens from the overlapping ranges of *V. Baldwini*

and *V. interior* are frequently intermediate in character. *V. aborigina* Gleason, known so far only from the original collection in southeastern Oklahoma, appears to be a giant form of *V. Baldwini*. It retains most of the morphological characters of that species, but is much larger in all its dimensions, with about twice as many flowers in each head. *V. missurica* Raf., the last of the group, has the widest distribution of the four, ranging from Texas, where it is not particularly common, north and east to Michigan, and becoming exceedingly abundant in Iowa, Illinois, and Indiana. It is characterized by larger, more compact inflorescence, fully differentiated pappus, and regularly imbricated involucre. Many specimens from the southern part of its range retain the sharply acute, relatively narrow involucral scales of *V. interior*, while those from farther east have fewer, broader, and obtuse or apiculate scales. The species also occurs to a limited extent and with slightly different structure along the Gulf Coast as far east as Alabama.

The origin of two other western species is in doubt. *V. Bolleana* Sch.-Bip., of northwestern Mexico, seems to bear no close relation to any other known species. *V. crinita* Raf., of the Ozarkian region, is characterized by filiform involucral scales, and may represent an extreme development from the Interiores.

The eastward migration along the coastal plain from Texas led to the present development of seventeen species. They are not easily divided into distinct species-groups, a feature possibly indicative of recent immigration and evolution. The most primitive group is the Angustifoliae, ranging from Louisiana east to the Atlantic, thence north to the Carolinas and south into Florida and the Bahamas. The group retains the primitive involucre, narrow leaves, and low stature of the Texanae, and the type species of that group was originally described as a variety of *V. angustifolia* Michx. Some of the species have an inflorescence approaching umbelliform and rather suggestive of *V. liatroides* DC. or other species of northern Mexico. *V. angustifolia* has the widest distribution, almost coextensive with that of the group. The other four, each of restricted distribution and lacking the acuminate scales of the simpler species, seem to represent recent evolutionary developments. Of these, *V. Blodgettii* Small, in southern Florida, marks the re-entrance of the group into the tropics, and leads to the closely related *V. insularis* Gleason of the nearby Bahamas.

The group Pulchellae is obviously closely related to the Angustifoliae, as shown by narrow leaves and general vegetative habit, but differs in the absence of resin glands on the achenes and in the prolongation of the involucral scales into filiform appendages. The three species, *V. pulchella* Small, *V. recurva* Gleason, and *V. scaberrima* Nutt., are all of limited distribution in the coastal plain of Georgia and the Carolinas.

The species-group Glaucae lies generally to the north of the Angustifoliae and has probably been derived from it. Here the heads are larger,

the pappus is tawny or almost white, and the involucral scales are long-acuminate or almost filiform. The leaves are large in proportion to the height of the stem, and the greatest expanse of foliar surface is toward the base of the stem. While this feature is apparent in *V. glauca* (L.) Willd., an Alleghenian species ranging northward to Pennsylvania, it is still further developed in *V. acaulis* (Walt.) Gleason and *V. georgiana* Bartlett, two coastal plain species with distinctly basal leaves.

Two other species with prolonged filiform scales constitute the group Noveboracenses. *V. noveboracensis* (L.) Michx. has attained a wide distribution over the Piedmont region of the eastern states from Mississippi to Massachusetts, occasionally invading the coastal plain also. There it has given rise to a localized species, *V. Harperi* Gleason, characterized by larger heads with more numerous flowers.

V. gigantea (Walt.) Britton is closely related to *V. concinna* Gleason, of the Angustifoliae, and like that species is confined to the southeastern portion of the coastal plain.

The last species-group of the southeastern states is the Altissimae. *V. ovalifolia* T. & G. is a variable species of the southeastern coastal plain, and appears to be the most primitive species of the group in their evolution from the Angustifoliae. Although some of its variants approach *V. concinna*, it is generally distinguished by the broader, regularly imbricated involucre and the broad leaves. *V. flaccidifolia* Small is a well-marked species of the southern Appalachian region. *V. altissima* Nutt., the last species of the group, has a wide distribution from Georgia and Alabama north and west to New York and Missouri. Typical forms of the species avoid the coastal plain and are characteristic of the woodlands of the central states, but the variety *laxa* Gleason occurs along the Gulf Coast. In its western extension *V. altissima* comes in contact with several species of the western migration route, and many intermediate forms occur which are probably to be considered as hybrids.

Considering the 35 species with paniculate inflorescence as a whole, we see that the species with primitive involucre invariably lie far to the south or southwest, and that those with the broadest and most obtuse scales, as well as those with the most filiform scales, lie always well to the north or northeast. It is also worthy of note that only seven of the 35 have attained a wide distribution, while the other 28 occupy small or localized ranges. These seven are *V. fasciculata* Michx., *V. interior* Small, *V. missurica* Raf., *V. angustifolia* Michx., *V. glauca* (L.) Willd., *V. noveboracensis* (L.) Michx., and *V. altissima* Nutt., representing six species-groups. This fact alone may indicate the comparatively recent immigration and incomplete evolution of the genus in the northern portion of its range.

The general relations of the 63 species of the genus in which the bracteal leaves are suppressed is exhibited in a diagram (fig. 3).

In the evolution of the genus in North America, no general plan or

tendency is apparent, except in the one feature of inflorescence. In every case it is found that species structurally aberrant from the general type

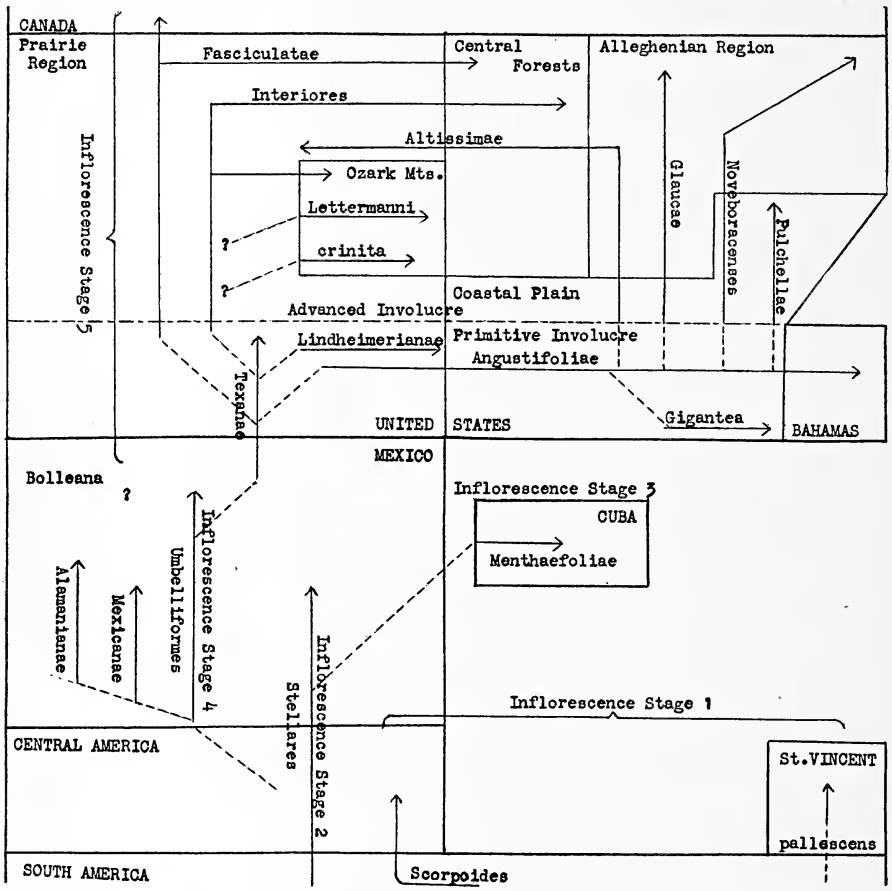

FIG. 3. Migration and evolution of the bractless Vernoniae of North America. Solid lines show distribution by their location, migration by the direction of the arrow. Dotted lines show probable connection between species-group.

occupy outlying ranges or peculiar habitats. The evolution in structure may be summarized as follows:

1. From elongate cymes to short, compact, freely branching cymes or capitate clusters.

2. From small, acute or acuminate involucral scales to broad, blunt, or veiny scales, or to narrow, prolonged, or filiform scales.

3. From a medium number (13–21) of flowers in each head to a large number (55–89, or even more), or to a reduced number (as low as 5).

4. From lanceolate, acuminate leaves to linear, 1-nerved, or revolute leaves, or to blunt, broad, rigid or coriaceous leaves.

In the leafy-bracted forms, comprising 57 species, there is a general

tendency toward the congestion of the inflorescence by repeated branching or toward its reduction by shortening the cymes. The former is most apparent in the species-group Arborescentes, the latter in the Racemosae and Acuminatae. There is a great reduction in the number of flowers in the outlying members of the Acuminatae, Sagraeanae, and Racemosae. Specialization of the involucre by the development of broad, blunt scales occurs in the Schiedeanae, and of narrow, prolonged scales in the Sagraeanae, while the Buxifoliae have increased the number and the regularity of imbrication of the scales. Leaves have shown a tendency to become broad and blunt from the Arborescentes through the Longifoliae and into the Bahamenses, or narrow, one-nerved, and revolute from the Arborescentes into the Racemosae. Montane species have been developed in the Acuminatae and the Buxifoliae, and in both cases are characterized by crowded, few-flowered heads and by small and broad leaves.

Among the 63 species with bractless cymes there is less diversification in structure, except in the inflorescence, which has already been discussed. In the Stellares there is a gradual progression from narrow, acuminate involucral scales to short and blunt ones. A similar tendency occurs among the Interiores and Altissimae, and reaches a climax in the series from the Umbelliformes to the Alamanianae and Mexicanae, with their highly specialized, broad or veiny scales. On the other hand, there is a notable tendency toward prolongation of the scales in *V. crinita* and in the Glaucae, Pulchellae, and Noveboracenses. Excepting the Stellares, all these groups show likewise a tendency to larger heads, reaching a maximum in the Mexicanae, Alamanianae, and *V. crinita*. Forms with unusually small heads rarely occur, and are most characteristic of the single species *V. gigantea*. Two groups only have developed montane forms, the Alamanianae and Mexicanae, and in their unusually large, many-flowered heads differ remarkably from the montane forms of the West Indies.

Neither is there any correlation between structure and habitat. The variation between the montane species of the West Indies and Mexico has already been mentioned. The relatively arid conditions of the Bahamas are reflected in the thick, firm leaves of the Bahamenses and *V. insularis*, but those of the former, with broad-leaved ancestors, are broad and blunt, while the latter, originating from the Angustifoliae of the Gulf States, preserves the linear leaves. The Racemosae, of arid situations in Cuba and Hispaniola, and *V. texana*, likewise a xerophyte, have narrow leaves, but the hydrophytic *V. fasciculata* has narrow leaves also, while the xerophytic *V. Baldwini* has broad leaves. The xerophytic Bahamenses have assumed the form of bushy shrubs, while *V. texana* has remained an herb, although growing in a region where the shrub form is common.

Three processes seem to have been concerned in the general history of the genus, by which it has reached its present distribution and differentiation. Physiological evolution, scarcely indicated by structure, has enabled the

genus to migrate into new environments beyond its original home; migration has brought it to its present distribution and is doubtless still continuing; structural evolution, favored by geographic isolation, has differentiated the present species, but is very little correlated with their physiology or ecology, although proceeding simultaneously with their migration. A single structural tendency appears to be general and possibly orthogenetic, that of the shortening and branching of the cymes.

Summary

1. Three sections of the genus are represented in North America by a single species each; one section is represented by 120 species.

2. In this section the chief differentiation of groups rests on the structure of the inflorescence; minor differentiation of species-groups is based on the achenes, the involucre, the pappus, and the character of the pubescence.

3. Of the two subsections, one is chiefly Antillean, the other continental, while both are developed in continental South America.

4. Characters which are held to represent primitive conditions in one group may indicate advanced evolution in another, and such characters have no apparent correlation with environment.

5. In every case, those groups which appear to be the simplest in morphological structure occur to the south, while the more complex groups appear progressively farther to the north. In most groups the same statement holds for the individual species.

6. The geographical arrangement of the species-groups and species follows well-known migration routes and supports the conclusion that evolution and migration have proceeded together.

New York Botanical Garden

THE AVAILABILITY OF IRON IN NUTRIENT SOLUTIONS FOR WHEAT[1]

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The efficiency of the usual trace of iron when employed in culture solutions may be expected to vary with the nature of the compound in which it is supplied, with the composition and reaction of the solution in which it is employed, and with the species of plant. Gile and Carrero have shown that the reaction of the nutrient solution (whether acid, neutral, or alkaline) (6), as well as soil conditions (7), have a marked influence upon the availability of iron to the rice plant. Corsan and Bakke (4) count ferrous iron less efficient than ferric iron, when used in the forms of phosphates. Jones and Shive (9) have pointed out that, in the nutrient solution which they employed, iron in the form of ferrous sulphate was very readily available to the wheat plant, but was evidently somewhat toxic in the highest concentrations used. Ferric phosphate, on the contrary, was very slowly and difficultly available to these plants, even when supplied in relatively large quantities. In later work (10) they found that ferrous sulphate was superior to ferric phosphate as a source of iron for plants grown in a nutrient solution containing calcium nitrate. When ammonium sulphate was employed as the source of nitrogen, however, the solution increased in acidity and ferric phosphate supported growth of the plants better than did ferrous sulphate. Hoagland (8) has suggested that the presence of sufficient dissolved iron in the culture solution will depend upon the form and quantity of the iron salt used, and considers iron citrate and tartrate the most effective forms. Duggar (5) has recommended a special form of "soluble" iron prepared from ferric citrate and sodium phosphate.

Experimentation

Solubility

In the present investigation an attempt was made to determine which of the following salts of iron, namely: ferric citrate, $FePO_4$, $Fe_2(SO_4)_3$, and $FeSO_4$, would remain in solution in the greatest amounts at the two hydrogen-ion concentrations of pH 4.2 and pH 6.0, using the Livingston-Tottingham nutrient solution; and also to determine which of these forms of iron would be most available to the wheat plant when supplied in varying amounts, at the latter pH value of the nutrient solution.

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station. This investigation was supported by a grant from the research funds of the University of Wisconsin.

Solubility of Iron Salts

Duplicate one-liter portions of the nutrient solution were prepared from the special salts provided for collaborators (3). To one portion was added enough M/2 KOH to increase the pH value from 4.2 to 6.0. To each of four aliquot portions of the solution at each pH value were added the equivalent of ten milligrams of iron in the form of one of the following compounds: ferric citrate, $FePO_4$, $Fe_2(SO_4)_3$, and $FeSO_4$. The solutions were allowed to stand for 24 hours at a temperature of about 22°C. They were then filtered and evaporated to a volume of about 100 cc. After oxidizing by boiling with the addition of HNO_3, the ferric iron was precipitated from the hot solution with NH_4OH. A white, flocculent precipitate was obtained in each case. The precipitate was filtered and washed until free from nitrate.

As the amount of iron present in each case was very small, an attempt was made to use the colorimetric method of determining the iron as sulfocyanate (1). Probably because of interference by the salts of the nutrient solution, the results were unsatisfactory.

The following procedure was therefore employed: After dissolving the precipitate in dilute H_2SO_4, the iron was reduced by the use of metallic tin and finally titrated with potassium permanganate. The results obtained by this method were fairly satisfactory. The agreement of duplicate solutions may be ascertained from the data given in table 1.

TABLE 1.

TABLE 1. *Solubility of Iron in Livingston-Tottingham Solution R_8C_1 when Amounts of Salt Equivalent to 10 Milligrams of Iron per Liter were Employed*

pH	Ferric Citrate	$FePO_4$	$Fe_2(SO_4)_3$	$FeSO_4$
	Mg. Fe per l.	Mg. Fe per l.	Mg. Fe per l.	Mg. Fe per l.
4.2.....	5.7	2.3	5.6	8.6
4.2.....	6.6	2.3	5.6	9.2
6.0.....	5.7	2.3	4.7	1.7
6.0.....	6.3	1.7	4.5	1.4

The outstanding feature of these data is the fact that ferric citrate is as soluble in the nearly neutral solution as in the more acid one. $FeSO_4$ is actually the most soluble form of iron at pH 4.2, but its solubility is depressed greatly when the pH value is increased to 6.0. $Fe_2(SO_4)_3$ compares favorably with ferric citrate at pH 4.2, and is not greatly depressed in solubility at pH 6.0. $FePO_4$ is relatively insoluble at both pH values.

Availability of Iron Salts in Solution Cultures

A series of cultures were arranged to compare the availability of organic and inorganic sources of iron, namely, ferric citrate and $Fe_2(SO_4)_3$; and also

to compare the ferrous with the ferric form of iron, namely, $FeSO_4$ and $Fe_2(SO_4)_3$.

The culture solution used in this experiment was Livingston and Tottingham's R_8C_1. The iron was supplied in three different planes as either ferric citrate or $Fe_2(SO_4)_3$, at rates equivalent to 2, 10, and 50 milligrams of iron per liter of nutrient solution. A comparison of $FeSO_4$ and $Fe_2(SO_4)_3$ was made only at the plane of application of 10 milligrams of iron per liter. Six replicative cultures containing five seedlings each were conducted for each plane of iron. A pure strain of Marquis wheat furnished by the Department of Agronomy of the University of Wisconsin was employed.

The method of germinating was as follows: The seeds were immersed in a solution of 0.1 percent mercuric chloride for fifteen minutes, and washed thoroughly with distilled water. They were then soaked in distilled water for 5 to 6 hours, after which they were spread upon mosquito netting which had been paraffined and stretched over granite-ware pans. The latter were 28 cm. in diameter and 10 cm. deep. They were placed in a bath supplied with tap water for maintaining a temperature of about 25°C. Nutrient solution was supplied continuously from a common reservoir at the rate of about 24 liters per pan per 24 hours. The medium employed was Livingston and Tottingham's solution R_8C_1, diluted to 1/10 its usual concentration and adjusted to a pH of 7.5 with M/2 KOH. When the

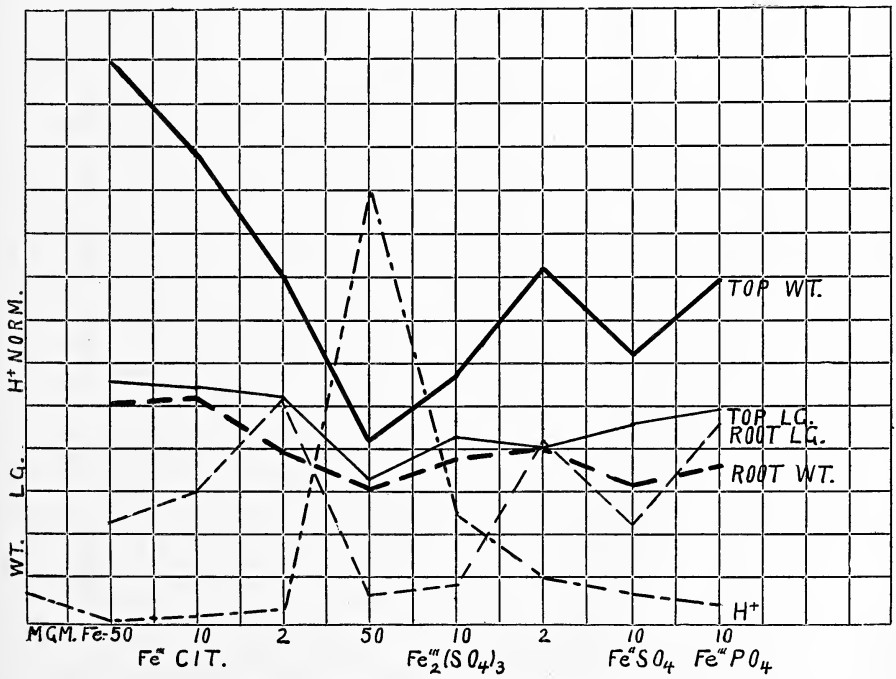

FIG. 1. Relations between sources of iron, acidity of neutral solution, and growth o wheat.

seedlings were about 10 cm. high, specimens of uniform height were selected for use. These were mounted in wide-mouthed jars of about 960-cc. capacity (quart Mason jars), covered with heavy paper so as to exclude light and restrict the growth of algae. The cultures were supported on rotating tables for the purpose of equalizing the exposure of all to variations of light and temperature. This procedure is explained in the manual for collaborators (3).

The experiment continued from April 14 to May 2, 1922. No records were taken of climatic conditions within the greenhouse. However, the thermostat of the house was adjusted for 15.5° C. night temperature. The conditions of solar radiation by day may be considered from the following data derived from records of the local weather bureau station:

Daily percentage of sunshine:
Max. 100.0, Min. 0.0, Mean 58.3.
Daily calories per sq. cm. per hour:
Max. 674.0, Min. 49.0, Mean 472.6.

The pH values of the solutions with various amounts of iron added were determined in the manner described by Clark (2). These values appear in table 2.

TABLE 2. *Values of Solution R_8C_1, pH Value 4.2, with Various Forms and Amounts of Iron added*

	Ferric Citrate			$Fe_2(SO_4)_3$			$FeSO_4$	$FePO_4$
Mg. Fe per l.	50	10	2	50	10	2	10	10
pH	5.0	4.8	4.4	3.0	3.6	4.0	4.2	4.4

From these data it appears that $Fe_2(SO_4)_3$ increased the hydrogen-ion concentration of the nutrient solution while ferric citrate decreased it. The pH value of certain solutions was determined also after the growth of plants over the 3-day period ending April 28. It was found that, when the two higher planes of $Fe_2(SO_4)_3$ were added, the pH value increased from 3.0 to 3.2 and from 3.6 to 4.0 for the 50-milligram and 10-milligram planes respectively. In all other cases the change of pH value was inappreciable. The plants were so small, however, that they could hardly be expected to exercise much effect upon the composition of the nutrient solution. Certainly, as regards form and appearance of the roots, the increased acidity from the use of $Fe_2(SO_4)_3$ was injurious to the plants.

Photographs of selected cultures were taken on May 1. These are reproduced in figures 2–4. On May 2, after 18 days of growth, the cultures were harvested and the usual separation of tops from roots was accomplished. The data of growth measurements are given in table 3.

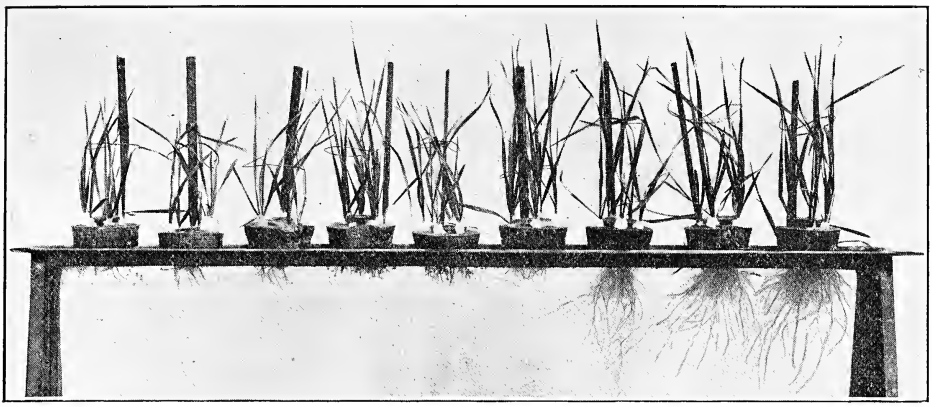

FIG. 2. Cultures 1, 2, 3, $Fe_2(SO_4)_3$, 50 mg. Fe per liter. Cultures 4, 5, 6, $Fe_2(SO_4)_3$, 10 mg. Fe per liter. Cultures 7, 8, 9, $Fe_2(SO_4)_3$, 2 mg. Fe per liter.

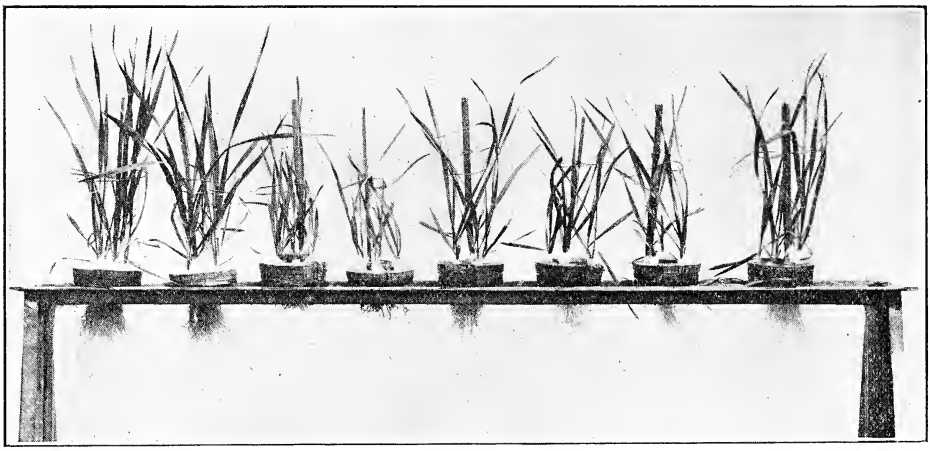

FIG. 3. Cultures 1, 2, ferric citrate, 10 mg. Fe per liter. Cultures 3, 4, $Fe_2(SO_4)_3$, 10 mg. Fe per liter. Cultures 5, 6, Fe SO_4, 10 mg. Fe per liter. Cultures 7, 8, Fe PO_4, 10 mg. Fe per liter.

FIG. 4. Cultures 1, 2, 3, ferric citrate, 50 mg. Fe per liter. Cultures 4, 5, 6, ferric citrate, 10 mg. Fe per liter. Cultures 7, 8, 9, ferric citrate, 2 mg. Fe per liter.

TABLE 3. *Data of Growth of Wheat in Solution R_3C_1, with various Planes of Added Iron*

Source of Iron	Length of Tops in mm.	Length of Roots in mm.	Weight of dry Tops in mg.	Weight of dry Roots in mg.	Comments
Ferric citrate, 50 mg. Fe per l.	223 ± 6*	92 ± 25	517 ± 43	280 ± 57	Tops dark green, roots yellowish and thick.
Ferric citrate, 10 mg. Fe per l.	221 ± 31	121 ± 13	433 ± 85	206 ± 21	Tops dark green, roots white.
Ferric citrate 2 mg. Fe per l.	209 ± 13	206 ± 25	321 ± 50	157 ± 26	Tops dark green, older leaves dead, roots white.
$Fe_2(SO_4)_3$, 50 mg. Fe per l.	133 ± 13	25 ± 10	168 ± 32	123 ± 56	Tops yellowish green between fibro-vascular bundles, many older leaves dead, roots thick and blackened.
$Fe_2(SO_4)_3$, 10 mg. Fe per l.	171 ± 13	31 ± 6	229 ± 70	153 ± 28	Tops light green, roots yellowish.
$Fe_2(SO_4)_3$, 2 mg. Fe per l.	161 ± 13	167 ± 13	328 ± 63	162 ± 28	Tops dark green but paler than those of citrate-iron cultures, roots white, numerous laterals evident.
$FeSO_4$, 10 mg. Fe per l.	184 ± 12	90 ± 6	250 ± 31	125 ± 5	Tops pale green, roots white, numerous laterals evident.
$FePO_4$, 10 mg. Fe per l.	195 ± 12	183 ± 13	315 ± 70	144 ± 23	Tops dark green, older leaves dead, roots white.

* Average departure from the mean of six cultures.

DISCUSSION

The results of the solubility test here reported show decided differences between the various forms of iron employed. It was found that $FePO_4$, a form commonly employed in nutrient solutions, was relatively insoluble at both pH values tested. $FeSO_4$, while the most soluble of the forms compared at the higher hydrogen-ion concentration, was relatively insoluble at the lower one. This result at the lower hydrogen-ion concentration would seem to indicate hydrolysis with the formation of insoluble $Fe(OH)_3$ or basic ferric salt. McCall and Haag (12) have called attention to this factor in the availability of iron in nutrient solutions. While $Fe_2(SO_4)_3$ compares favorably with ferric citrate in solubility, it increased the hydrogen-ion concentration of the nutrient solution to what seems to have been an unfavorable extent. It was also slightly depressed in solubility in the less acid solution. Ferric citrate appears to possess a fair degree of solubility over a considerable range of pH value of the nutrient solution. With the

ated with high concentrations of potassium di-hydrogen phosphate and consequent high hydrogen-ion concentration. Excessive branching of roots and stimulation of the growth of root hairs have been observed by Hoagland ('17), although he does not mention the characteristic stubbiness.

This injury to the roots, which antedates injury to the tops, conceivably and apparently renders the roots unable adequately to perform their functions, and presumably thus causes the later decreased growth of tops observed by other writers. Since the substances in the nutrient solution, as they pass into the plant, encounter the root hairs first, the effect upon the protoplasm of these cells was investigated, and the problem was accordingly narrowed at this point in the investigation to the changes that take place in root hairs.

Protoplasm consists, at least chiefly, of substances in the colloidal state. This has been known ever since the dark-field microscope came into use among biologists, although the precise nature of the colloids is as yet undetermined. Gaidukov ('10) described protoplasm as a sol because he observed many characteristics—for example, Brownian movement—that suggested a liquid condition. Since this pioneer work was published, however, studies of a greater variety of cells, and of the same cell under different external conditions, have shown that protoplasm is not to be so briefly characterized. Seifriz ('20) disagrees with Kite ('13) in his belief that protoplasm is ever so rigid that it can be cut into pieces that do not change in shape, and states that its cohesion is never greater than that of a plastic and viscous jelly. He found by micro-dissection that the streaming protoplasm of Rhizopus is fluid, while the endoplasm of Amoeba is decidedly viscous. After experimenting with protoplasm from many kinds of cells, both plant and animal, he concludes that within a given cell the viscosity decreases as the protoplasm becomes more active, and increases as it becomes more quiescent. This phenomenon had already been observed by Price ('14), who noted that the protoplasm of Mucor spores changes from jelly to sol as the spores germinate, and that the protoplasm of Fucus eggs changes from sol to jelly as the eggs mature. The general conclusion to which the work of these and other investigators points is that the viscosity of protoplasm is not constant; it is different in different cells, it may be different in the same cell at different times, and, indeed, it is highly probable that it is different in different parts of the same cell at the same time.

Chemically, protoplasm probably consists of a "complex of substances of various chemical natures and in various states of aggregation, associated by forces of surface tension, electrical charge, and so forth."[3] The exact nature of this complex is as yet undetermined; Lepeschkin ('13) believes it to consist largely of proteins and lipoids, while MacDougal and Spoehr's ('20) "bio-colloids," which simulate in many ways the colloidal behavior of protoplasm, are mixtures of proteins and pentosans, and are greatly in-

[3] Bayliss ('18), p. 26.

fluenced by amino acids. It may be that there are two essentially different substances in protoplasm, one more viscous than the other, and that various substances are distributed in these two media as non-living inclusions. It is also possible that living protoplasm exists in only small quantities in any cell, while the greater share of the substance now termed protoplasm is composed of products of the cell enzyms; products which are themselves non-living, but which, because they may change their colloidal state readily, may often appear as the conspicuous part of the entire system. Coagulation and variations in viscosity observed in "protoplasm" might thus be due to colloidal changes in non-living inclusions. Whatever value these suggestions may have, the data are at present insufficient to decide between them. Irrespective of just what it is that coagulates, coagulation is a sign that the cell is becoming more inactive. For the descriptive purposes of this paper, "protoplasm" will be considered to mean the entire visible colloidal complex. It varies in viscosity from a sol to a stiff jelly, and it contains or is associated with proteins, carbohydrates, fats, lipoids, and salts, in unknown combinations. Because it can form jellies, and because more than a trace of electrolyte is required to precipitate it, it is classified with the emulsoids.

The part that is played by the surface layer of a protoplast in determining the permeability of protoplasm is not known. By some writers it has been regarded as a membrane of peculiar composition; by others as merely a condensation film of protoplasm, differing from the interior of the protoplast in its surface-tension qualities. Of course the latter hypothesis cannot hold, for the Gibbs-Freundlich law, which states that substances that lower the surface tension of a system become more concentrated at the surface, and that substances that raise the surface tension become more concentrated in the interior, applies to solutions only. Protoplasm is obviously not a solution, and we have no knowledge of the laws that govern the distribution of substances in a heterogeneous, colloidal system. Whatever the nature of the surface layer may be, it is to be remembered that it is possible for a substance in solution to affect the structure of a protoplast whether it enters it or does not enter it. If it enters, its effect will be determined by the nature and amount of the substance, the condition of the protoplasm, and the natures and relative amounts of the substances in the protoplasmic layer and in the cell sap. It might penetrate the protoplast without changing it; it might alter the colloidal structure by reacting with the constituents of the protoplasm or merely of its surface layer; or it might change the electric signs of the colloids. If the substance does not enter the protoplast, it might react with substances in the surface layer, or by adsorbing ions it might change the electric signs of the colloids, and thus affect the colloidal structure without having entered the protoplast. It is apparent, then, that the terms "permeability" and "impermeability" are applied rather loosely to the perceptual results of several different processes. Physiologists have not yet been able to determine for given

situations just what processes actually operate. Indeed, unless a substance is so colored that its presence can be actually seen in the protoplast, or unless its passage can be traced through the plant, the experimenter does not even know whether or not the substance entered the protoplast of which the structure is affected. In order to determine what process operates in a given situation, he must know just how the given substance affects the given protoplast. Very little of this kind of work has been done.

The dark-field microscope offers the best method for this kind of study, because it makes possible observation of colloidal particles which are too small to be resolved by the ordinary microscope. The apparatus used in the present investigation consisted of a compound microscope fitted with a Zeiss cardioid condenser, a 1.8-mm. achromatic objective, and a 10 x ocular. The cardioid condenser requires intense illumination because the cone of light becomes very broad before entering the objective. A small arc lamp with carbons fixed at an angle of 70° was found satisfactory. The light was passed through an aqueous ammoniacal solution of copper sulphate to remove the red rays, and was then focused on a plane mirror, from which it was reflected into the condenser. The slides and cover glasses were selected for a uniform thickness of 1.0 and 0.1 mm. respectively. They were cleaned with alcohol, dipped in collodion, dried, and then stored in water; immediately before a slide or a cover glass was used, it was wiped free from water and the thin film of collodion was stripped from the surface, thus removing all dust particles.

The protoplasm of an actively growing root hair appears milky under the dark-field microscope because the particles are so small, so numerous, and so evenly distributed that the enlarging light cones overlap, thus making it impossible to distinguish the individual particles. In a very young root hair the protoplasm is dense and almost devoid of vacuoles. As the root hair grows, the protoplasm becomes less dense, vacuoles form and enlarge, and the cell is apparently at the height of its usefulness as an absorbing organ. The vacuoles continue to enlarge and begin to coalesce, and the protoplasm is crowded more and more toward the outside of the cell, so that finally it is but a thin film separating the cell sap from the cell wall, and the root hair is of little value to the plant. To study the effects of nutrient solutions on the protoplasm of root hairs, it is thus obviously necessary to select cells that are in the second stage of their grand period of growth, for at this age their condition determines their value to the plant.

Root hairs of wheat grown in the nutrient solutions described above differ markedly in the appearance of their protoplasm, when they are examined at the age of approximately maximum usefulness to the plant. These differences are shown in figure 1, Plate XXIII. In hairs from plants grown in solution 1 (pH 3.94) the protoplasm is evenly distributed through the cell and shows no suggestion of precipitation [4] or of aggregation into masses.

[4] To avoid ambiguity, it becomes necessary to define the following terms, which have been used in different senses by different colloid chemists:

Hairs from plants grown in solutions 2 and 3, of which the hydrogen-ion concentration is higher (pH 3.85 and 3.68 respectively), contain more vacuoles, but there is no indication of coagulation. Decided differences are apparent between the protoplasm of root hairs grown in solutions 4 and 5, which are still more acid (pH 3.60 and 3.47 respectively), and that of the root hairs mentioned above. Hairs from solution 4 show the beginning of gel-formation: a coagulum has begun to form, the dispersion has decreased sufficiently to enable one to distinguish individual particles, and these are becoming flocculated into larger masses; however, there are still large portions of the protoplast that do not show coagulation. In hairs from solution 5, gel-formation is more pronounced: coagulation and flocculation are evident throughout the protoplast. In some places the flocculent masses are collected in the interior of the cell, thus indicating that the root hair is not turgid and is consequently of no value as an absorbing organ. Some protoplasm is still unprecipitated, but the amount is so small that it is almost imperceptible because of the contrast between the dark background and the white, flocculent masses of irreversible gel.

As has been noted above, this gel-formation occurs in the root hairs taken from cultures that have a large phosphate content, and consequently a large hydrogen-ion content, and it does not occur in root hairs grown in solutions containing a smaller proportion of the phosphate. Since the calcium-magnesium ratio is the same in all these solutions (except solution 3, which, although it differs slightly in calcium-magnesium ratio, was included in the present experiments because it is generally considered Shive's "best" solution), and since there is no evidence to show toxicity on the part of any of the other ions in these solutions, the conclusion that the gel-formation is due to the hydrogen ion seems inescapable.

This correlation is further supported by experiments that were performed to determine the effect of acids on protoplasm. In these experiments the hydrogen-ion concentration was varied, not by changing the salt proportions in nutrient solutions, but by irrigating the root hairs with solutions of an inorganic acid in pure water. The seeds were germinated on porous pottery kept moist with tap water, and the plants were taken directly from the pottery germinators for study when they were two or three days old. In this drier habitat they produced an abundance of root hairs. Moreover, the protoplasm in these root hairs was apparently denser, for under the dark-field microscope they appeared whiter. The osmotic pressure of the root hairs was also increased, for they were not plasmolyzed by a 0.45 M solution of hydrochloric acid, sodium chloride, or nitric acid.

A *gel* is a solid formed from a sol or a jelly by the action of heat or of chemical reagents, *i.e.*, by processes other than mere loss of water. It is irreversible because the sol or jelly state cannot be regained by addition of water.

Precipitation or *coagulation* is the formation of a gel. It may be accompanied with a decrease or an increase in the dispersion of the particles.

Flocculation is the aggregation of precipitated particles into large, soft masses that remain suspended in the medium.

The roots, with root hairs, were mounted under the dark-field microscope in aqueous solutions of acid, and were irrigated with the same solution from time to time. Figure 2 shows successive stages in the precipitation of the protoplasm of one of these hairs that was subjected to the action of a 0.45 M solution of hydrochloric acid during a period of five hours. The similarities in appearance of this hair and of those previously described should be noted, especially since in this hair successive stages in the reaction between the hydrogen ions in relatively high concentration and the protoplasm of a single hair is being followed, whereas the drawings in figure 1 show different root hairs that were acted upon by hydrogen ions in different concentrations. Coagulation appears first at the vacuolar membranes and spreads through the protoplasm, enlarging the vacuoles as flocculation proceeds. The process is identical with that in the root hairs that were grown in nutrient solutions containing a large amount of phosphate, except that the flocculi are larger and the dispersion medium is clearer when the precipitation is affected by the hydrochloric-acid solution. Quantitative differences are to be expected, for the hydrogen-ion concentration is higher.[5] Moreover, in the nutrient solutions two processes may be considered to be operating—one induced by the nutrient ions, tending to maintain the normal structure of the protoplasm, and the other induced by the hydrogen ions, tending to coagulate it. In the hydrochloric-acid solution there are practically no nutrient ions, so that the coagulation by the hydrogen ions is not repaired. This so-called "antagonism" of ions is well known, although the processes affected are not always the same.

In order to determine whether or not coagulation was due to a specific effect of the hydrogen ion, the same experiment was performed with a 0.45 M solution of sodium chloride instead of hydrochloric acid. The results are shown in figure 3. At the end of five hours the protoplasm showed no change except that it had become slightly more vacuolated. Since the two equivalent solutions differ as to cation but not as to anion, it seems clear that the coagulation caused by the hydrochloric acid was induced by the hydrogen ion. This inference has led some investigators [McCall and Haag ('20), Meier and Halstead ('21)] to seek a direct relation between hydrogen-ion concentration and the yield of plants, but they were unable to find such a relation, although still convinced that the hydrogen ion does exert an influence on plant growth. The nature of this influence forms the subject of this paper, and it will emerge that this influence, although it is decidedly effective, is of such a kind that there can be no direct relation between the hydrogen-ion concentration of nutrient solutions and the yield of plants grown in them.

The conclusion that the coagulation of the protoplasm was induced by the hydrogen ion becomes still more probable when the close similarity in appearance between this cell and those protoplasts that were coagulated

[5] The pH of a 0.45 M solution of HCl is approximately 0.4.

in the culture solutions is noted, for protoplasm is coagulated differently by different reagents. For instance, osmic acid characteristically produces a net-like coagulum,[6] which differs materially from the flocculum observed in the present experiments.

Although the argument above presented seems logical, it may be that the coagulation was induced by the chlorine ion, and that the sodium ion rendered it innocuous when sodium chloride was used instead of hydrochloric acid. The experiment was accordingly repeated with a 0.45 M solution of nitric acid, the anion of which was formed in the nutrient solutions by the dissociation of calcium nitrate. The results, shown in figure 4, duplicate the results that were obtained with hydrochloric acid and with the nutrient solutions of high phosphate concentration. The conclusion seems inescapable that in every instance the coagulation is due to the hydrogen ion.

A further experiment was undertaken to determine whether or not the presence of salts alters the rate of coagulation by the hydrogen ion. Root hairs were irrigated with solution 2 to which sufficient nitric acid had been added to make the concentration of acid 0.45 M, and note was taken of the time required to produce a degree of coagulation equal to that produced by the end of four and one half hours when an equimolecular solution of nitric acid in pure water was used. Of the two root hairs irrigated with nitric acid in salt solution, the protoplasm of one was coagulated to the extent defined above in four hours, that of the other in five hours. Since these results indicate a pronounced individual difference in root hairs, and since the effect of salts on the chemical potential of the hydrogen ion is not properly a part of the present thesis, the experiments were discontinued. The experiment is cited, however, and drawings of one of these hairs are shown (fig. 5), because, since the coagulation was produced in a nutrient solution, and since it is of the same kind as that usually produced in solutions of high hydrogen-ion concentration, although to a greater extent because of the added acid, it affords additional evidence, if that be needed, that the coagulation in the culture solutions is due to the specific effect of the hydrogen ion.

It is now possible to explain the abnormal root development in those nutrient solutions that contain an injurious quantity of potassium di-hydrogen phosphate. The hydrogen ions precipitate the protoplasm of the root hairs, which are the primary absorbing cells of the plant, thus increasing their permeability and rendering them unable to act as absorption organs. As the root hairs become ineffective, more are formed, as in dry soils or in culture solutions such as solutions 4 and 5, where the phosphate concentration is too high. If the root cannot produce enough root hairs to carry on the work of the plant, which in turn becomes stunted, then more roots are formed, thus producing the short, branched root systems described above. This series of effects affords another[7] example of the Le Chatelier-Braun

[6] Bayliss ('18), p. 15.

[7] For other examples, see Bancroft ('11).

theorem [Le Chatelier ('84), Braun ('87)] ,which states that a system affected by an outside condition tends to alter within itself in such a way as to oppose and partially annul the effects of this outside condition.[8] It thus becomes evident that there can be no direct relation between the hydrogen-ion concentration of nutrient solutions and the yield of plants grown in them, for the hydrogen ion affects the absorbing action of the plant, which in turn affects, not any one plant measurement, but all directions of growth.

Summary

1. The abnormal root development and decreased growth that have been observed in plants grown in nutrient solutions that contain relatively large amounts of potassium di-hydrogen phosphate may be explained by the coagulation of the protoplasm of the root hairs.

2. This coagulation, which is accompanied with flocculation, is found to be induced by the hydrogen ions formed by dissociation of the phosphate. The hydrogen-ion concentration of the nutrient solutions employed varied from pH 3.94 to pH 3.47.

3. The relation of this coagulation and flocculation to the colloid chemistry of protoplasm is discussed.

4. The lack of logic in the attempts of certain investigators to find a direct relation between environmental features, such as hydrogen-ion concentration, and the dry weight of plants, is pointed out.

In concluding, I wish to express to Professor Howard E. Pulling of Wellesley College sincere gratitude and appreciation for many suggestions, constant interest, and other assistance during the progress of the experiments and the preparation of this paper.

Wellesley College,
Wellesley, Mass.

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[8] Chwolson ('05), p. 475.

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EXPLANATION OF PLATE XXIII

The optical apparatus used consisted of cardioid condenser (Zeiss), 1.8 mm. achromatic oil-immersion objective (Spencer), and 10 × ocular (Spencer). In the figures a camera lucida (Bausch and Lomb) was used in drawing the outlines and indicating the general regions of the cell content. Details were drawn free-hand. The magnification of the figures is approximately 810 diameters.

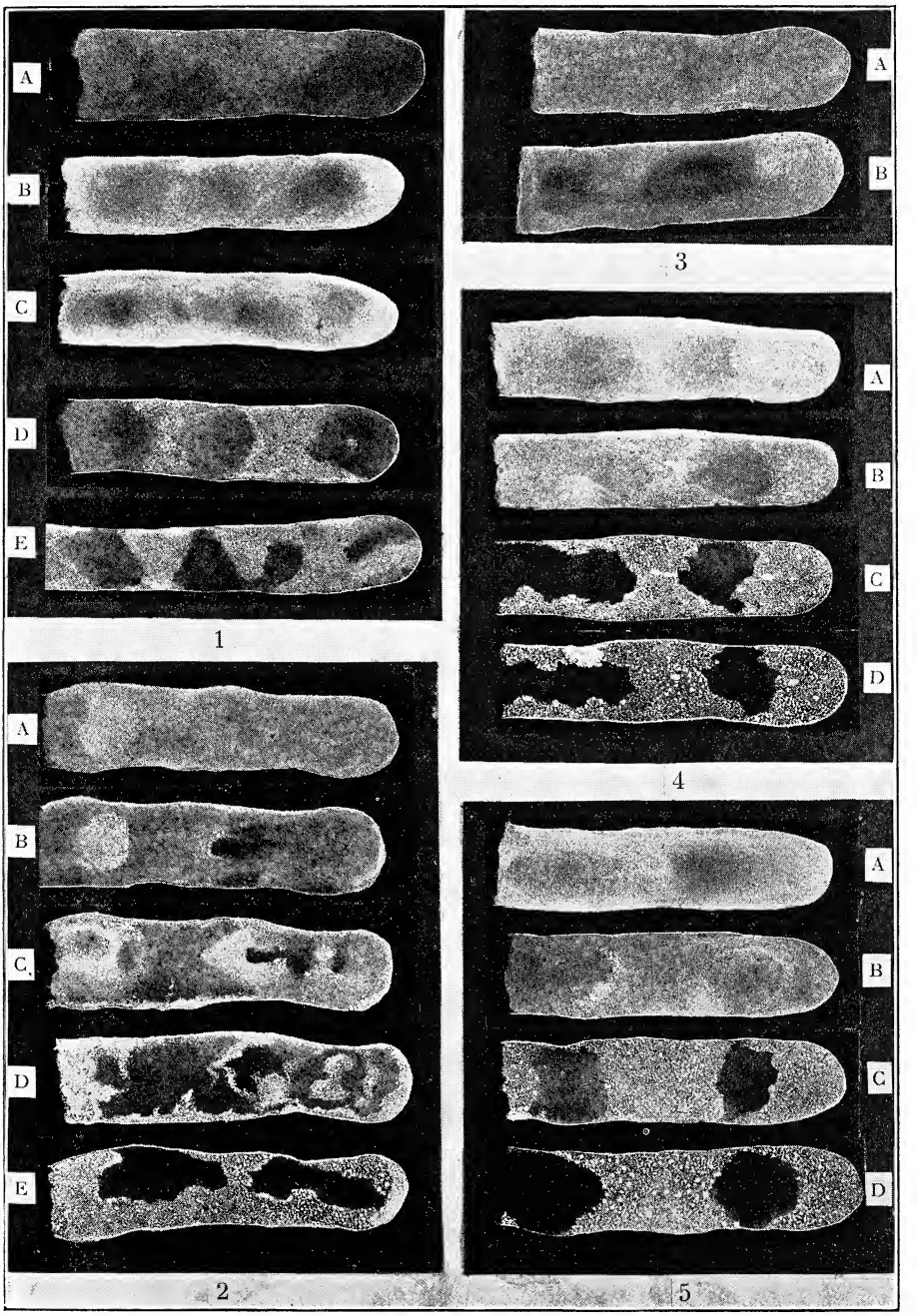
FIG. 1. Root hairs of wheat grown for one week in the following solutions: *A*, solution 1. *B*, solution 2. *C*, solution 3. *D*, solution 4. *E*, solution 5.

FIG. 2. Root hair of wheat, showing the effect on the protoplasm of a 0.45 M solution of hydrochloric acid in pure water. *A*, immediately after being mounted in the solution. *B*, at the end of ½ hour. *C*, at the end of 1½ hours. *D*, at the end of 2½ hours. *E*, at the end of 5 hours.

FIG. 3. Root hair of wheat, showing the effect on the protoplasm of a 0.45 *M* solution of sodium chloride in pure water. *A*, immediately after being mounted in the solution. *B*, at the end of 5 hours.

FIG. 4. Root hair of wheat, showing the effect on the protoplasm of a 0.45 M solution of nitric acid in pure water. *A*, immediately after being mounted in the solution. *B*, at the end of 1½ hours. *C*, at the end of 3½ hours. *D*, at the end of 4½ hours.

FIG. 5. Root hair of wheat, showing the effect on the protoplasm of a 0.45 M solution of nitric acid in culture solution 2. *A*, immediately after being mounted in the solution. *B*, at the end of 1½ hours. *C*, at the end of 4 hours. *D*, at the end of 5 hours.



ADDOMS: HYDROGEN ION AND PROTOPLASM

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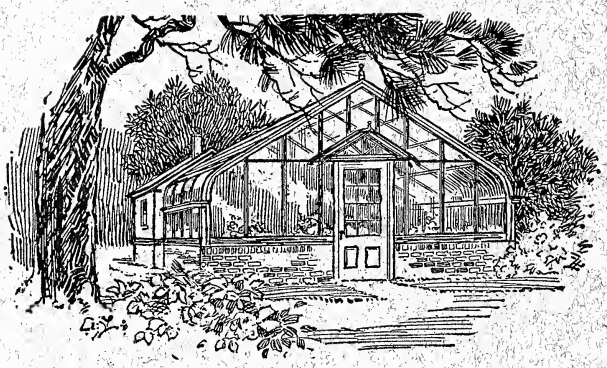

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THE SPECIES CONCEPT FROM THE POINT OF VIEW OF A GENETICIST[1]

GEORGE H. SHULL

Classification is a fundamental characteristic of scientific work in every field, and recognition of the fact that plants and animals may be described and discussed in group terms was doubtless the earliest manifestation of biological science. The advantages to every branch of biology of having group descriptions designated by brief names, which pass current in the language of all civilized peoples, is so manifest as to make the statement of the fact little more than a platitude. Indeed, the grouping of individuals into definitely limited categories whose names are common to all languages may be likened in its importance to the discovery of language itself. It provides the means of intercommunication, not only among biologists, but between biologists and humanity in general.

In order that this primary function of biological classification, as a basis of effective intercommunication, may be fulfilled, it is important that the limitations of the categories be such as may be capable of observation and comprehension by those among whom intercommunication is to be established or maintained. It seems, therefore, that the proper basis for classification is intimately related to the question of convenience. A consideration of all the different grades of classification: kingdoms, phyla, orders, families, genera, species, subspecies, forms, etc., etc., shows that the *species* represents the simplest concept, and it is therefore the one best adapted to serve as a vehicle of *general* communication.

Species may be defined as the easily recognized kinds of organisms, and in the case of macroscopic plants and animals their recognition should rest on simple gross observation such as any intelligent person can make with the aid only, let us say, of a good hand-lens. Genera, families, and higher orders of classification belong rather to biological philosophy, while the subspecific categories represent refinements which are of interest only or chiefly to specialists. In other words, *species* belong to biological "Main Street," and their chief usefulness lies in the very fact of the ease and definiteness with which they may be recognized.

[1] Read in the symposium on "The Utility of the Species Concept," at the joint meeting of Section G of the American Association for the Advancement of Science, the American Phytopathological Society, and the Botanical Society of America, at Toronto, December 28, 1921.

[The **Journal** for April (10 : 167–220) was issued April 18, 1923].

The geneticist has a peculiar interest in species and has always dealt with problems which have to do with the real inwardness of the species concept. Biological philosophers have often given genetical definitions of the species concept, as did the "Father of Botany" himself in a certain limited sense when he assumed that there are "as many species as the Infinite Being originally produced different forms."

The first great geneticist, J. G. Koelreuter, devoted a great deal of attention to an attempt to determine whether certain putative species were in reality specifically distinct or only varietally, basing his conclusions on the degree of sterility or fertility of the two forms under test, when bred together, and upon the fertility or sterility of the hybrids produced by such cross-breeding.

Not only this capacity for normal interbreeding, but also the degree of variability which occurs within normally interbreeding groups, have served as useful genetical characteristics of specific delimitation; but the usefulness of these phenomena as aids in determining what groups to call species is greatly lessened by the facts that so many species do not normally interbreed, and that not a few can not interbreed, as, for example, the parthenogenetic species of Taraxacum, Antennaria, etc.

These facts make it necessary, in the use of such a criterion of species, *merely to estimate* the amount of variation *which might be expected* to occur normally within a freely interbreeding group; and when we must resort to this sort of abstraction, the method at once loses its value in relation to the discovery of *natural* limitations of species. The fact is that, although genetical phenomena form the basis of nearly all biological classification, there is no *genetical* criterion—*nor any other criterion*—of specific difference, which is found generally applicable or generally acceptable. The geneticist comes more and more to the point of view that the distinctions between species are only quasi-natural, that specific differences are by no means quantitatively equivalent in different genera, families, orders, or phyla, but that genetically there are very many different grades of distinction between the form-groups which are, with more or less justification, recognized by taxonomists as "species."

This being the case, it seems proper to insist that utilitarian principles should be crucial in the establishment of new species and the maintenance of old ones. When we consider the whole question from the standpoint of convenience, it is clear that the needs and experiences of the user must be determinative.

To the systematist, whether professional or amateur, the species classification is the "bread of life"; but to other classes of biologists the species is merely a tool, handy or unhandy according as the taxonomist has done his work wisely or unwisely. To these other classes of biologists the species exist for the biologist, not the biologist for the species. Hence it often happens that the physiologist, the ecologist, the geneticist, etc., take

what doubtless seem to the taxonomist rather shocking liberties with the taxonomic species.

A simple example from my own work will illustrate how taxonomic distinctions may become useless to the geneticist. For a number of years I bred the dioecious forms of Lychnis (Melandrium) quite extensively, growing many hundreds of pedigreed families from seeds secured from various parts of Europe and America. I found that nearly every lot of seeds produced a progeny perceptibly different from those produced from other lots of seeds; but the differences, which were in some cases sharp and easily stated in precise terms, were in other cases difficult of exact description. All these forms—of whatever name and wherever collected—bred together without any diminution in fertility—in fact, usually with increased fecundity; and, although there were numerous differentiating hereditary characters, these were not grouped in the wild forms in such manner as to make it profitable or convenient to attempt to keep track of specific distinctions, as such, in the experimental garden. In my published accounts of these experiments the Linnaean designation, *Lychnis dioica*, was the only one which could be usefully employed, the English forms *L. diurna* and *L. vespertina* and the German forms *Melandrium album* and *M. rubrum* being incapable of maintenance.

Since the condition which here made the minor distinctions between taxonomic species useless was the perfect freedom with which all forms interbred, and the independence with which the hereditary characteristics were distributed, Koelreuter's criterion of specific difference receives support, and it might be assumed that under experimental conditions Koelreuter's method of delimiting species on the basis of compatibility or incompatibility —fertility *vs.* sterility—would have *general* genetical validity, except in the cases already mentioned in which vegetative or parthenogenetic methods of breeding occur; but any attempt to make extensive use of this idea promptly meets difficulties of most formidable dimensions on account of the numerous kinds and degrees of incompatibility and sterility which are met with.

Sterility may result from the presence of a single unit factor, of no more fundamental nature than the factor which changes a white flower to a colored one, or *vice versa*, in any one of a large number of species which might be mentioned. In such a case only a single gene differentiates the two forms, all the rest of the complex organization of the genotype being identical. On the other hand, incompatibility and sterility may result from general dissimilarities in organization, involving, conceivably, innumerable details of genotypic structure. I may cite several examples from my experiments with Bursa to illustrate the difficulties attendant upon any attempt to utilize incompatibilities and sterilities as *general criteria* of specific differences.

For fifteen years I have been working extensively with the common

shepherd's purse (*Bursa bursa-pastoris*) and to a less extent with several other species of the same genus. In connection with this work I have had material sent to me from nearly every part of the temperate regions of the world. Every new lot of material received has added to the demonstration that there occurs in nature an amazing number of hereditarily distinct forms within the species *B. bursa-pastoris*. While these biotypes are genetical entities of perfect delimitation, they can not by any method be given taxonomic validity as species or subspecies, because, under natural conditions, the genotypic factors prove to be less effective in determining the visible qualities which are necessarily utilized in classification by inspection than are the environmental factors. Even the larger features of leaf-lobing to which I have assigned the subspecific names *heteris*, *rhomboidea*, *tenuis*, and *simplex* are, oftener than not, quite indistinguishable in the field, and it has not been infrequent to have forms sent to me by good taxonomists, under the name "*integrifolia*," turn out to be *heteris*, the most highly lobed type. The significance of this is that the distinctive *genetical* characters are easily suppressed by crowding, poor soil, poor illumination, and other adverse conditions. The only practicable thing to do in a case of this kind is to maintain the species *bursa-pastoris*, recognizing its composite character, and in so doing to recognize also that species must be based upon characters whose existence is not too seriously affected by deviations in the environment.

In relation to the question of sterility of hybrids as evidence of specific distinctness in the parents, I may cite the following cases in *Bursa bursa-pastoris*, and its hybrids with *B. grandiflora* and *B. Viguieri*.

In 1913 I received from Professor A. H. Trow, of Cardiff, Wales, several packets of seed of Bursa specimens growing in close proximity to one another at Cardiff. Two of the progenies grown from these seeds differed slightly in outline of the capsules and in form and surface of the leaves, but neither suggested by its appearance that it ought to serve as the type of a newly defined species, and, indeed, it is doubtful whether they could have been separated at all when growing in nature. However, the two strains thus derived, when crossed together, gave hybrids which were almost completely sterile. When two types are thus found intermingled in nature and not capable of ready separation on inspection, they can not properly be referred to distinct species, even though their crosses show sterility; but, on the other hand, when we find that all up and down the Pacific coast of North and South America and extending eastward at least as far as Tucson, Arizona, and Waco, Texas, a characteristic form of Bursa occurs, having invariably concave-sided capsules, and yielding only sterile or almost sterile hybrids when crossed with numerous biotypes of *B. bursa-pastoris* from Europe and the eastern half of North America, which latter biotypes have prevailingly convex-sided capsules, one may with considerable justification urge the recognition of this west-American form as a species,

distinct from the species common to eastern America and Europe. In other words, it is *convenient* to differentiate between groups of organisms occupying different geographical regions, even though the differentiating characteristics are few and of relatively insignificant magnitude, while it is impracticable to separate *intermingled* groups differentiated by similar relatively slight characters.

A second kind of sterility is exhibited within the very distinct large-flowered species, *Bursa grandiflora* Bois., native of the regions about the Aegean Sea. In this species I have been able to demonstrate that there are two groups of plants, *A* and *B*, not visibly differentiable, both of which are sterile or nearly so when selfed or when crossed with other individuals of their own kind, but which are fully fertile when any two individuals mated together belong to different groups. In other words, $A \times$ self or $A \times A$, and $B \times$ self or $B \times B$, yield no offspring or almost none, while $A \times B$ or $B \times A$ is fully fertile and produces seeds abundantly. Here we are dealing with so-called self-sterility and cross-fertility; but, as already stated, there is also *cross-sterility* here, since *A* crossed with any other *A* plant or *B* with any other *B* plant produces no seeds. No one can maintain that incompatible crosses in such a case as this indicate specific distinctness between the parents, for *it is just the most closely related individuals*, those of like genotypic constitution, that are incompatible.

When we consider the results of crossing together such very distinct species as *Bursa bursa-pastoris*, *B. grandiflora*, and *B. Viguieri*, an even more striking limitation on hybrid sterility as an indication of specific distinctness is impressed. The wide differences in the three species here named may be noted in table 1, in which are entered their several contrasting characters.

TABLE 1

	Grandiflora	*Bursa-pastoris*	*Viguieri*
Rosettes	Lax	Rather lax	Dense
Leaves	Always well lobed	Variously lobed	But little lobed
Surface	Smooth, shining	Moderately smooth	Rugose, dull
Stellate hairs	Numerous	Abundant	Rare, small
Stems	Normal	Normal	Fasciated
Flowers	Large	Medium size	Small
Odor	Strong balsam-like	Absent	Absent
Breeding	Self-sterile	Self-fertile	Self-fertile
Carpels	2	2	3 to 8 (mode 4)

During the past six years I have made more than twenty hybridizations between *B. Viguieri* and *B. bursa-pastoris*, using in each case a different biotype of the latter species. Hybrids have always been easily secured from these crosses, the fecundity being at least approximately as great as when pollen from the same species has been used. The hybrids have always been vigorous plants of the *bursa-pastoris* type, no matter which

species played the rôle of mother in the cross, but these hybrids were always so nearly sterile that in no case was more than one seed found in any capsule of one of the F_1 plants, nor were more than two capsules, each with one seed, ever found on any one such plant, and only rarely did an F_1 plant have even one seed. At one time two F_2 plants were secured from these rare seeds, but these were lost before their adult characters had been observed. The remarkable distinctness of the two species, *bursa-pastoris* and *Viguieri*, left no ground for surprise at this F_1 sterility, and one would be inclined, perhaps, to say that *Viguieri* has deviated so far from the *bursa-pastoris* type, from which it doubtless took its origin, that it can no longer produce fertile progenies when back-crossed to the ancestral type.

When crosses are made between *B. bursa-pastoris* and *B. grandiflora*, a somewhat different relationship is indicated. Nearly twenty crosses of this type have also been made, but only in one case have F_1 hybrids (3) been secured, though the pollination usually results in the full development of the capsules and the ovules usually enlarge to nearly full size and then abort. Of three hybrids secured from one of these crosses, all were partially fertile, but fertility was slight in two, and in the third it was far from complete. From the most highly fertile of these an F_2 was grown which was so nearly sterile that it was not considered practicable to pursue the experiment farther.

The complete failure to secure F_1 hybrids from all but this one cross is in marked contrast with the easy production of F_1 hybrids in the *bursa-pastoris-Viguieri* crosses. Here again it may seem a logical explanation to say that *Bursa grandiflora* has evolved so many (or such considerable) divergences from the *bursa-pastoris* type that it can only rarely produce hybrids when crossed to the latter, thus giving seemingly good ground for the acceptance of such hybrid sterility as a criterion of specific distinctness.

But what becomes of the validity of this criterion when it is found that *Bursa grandiflora* and *B. Viguieri*, which give only sterile hybrids, or none at all, with the intermediately placed *B. bursa-pastoris*, *produce fully fertile hybrids when crossed with each other?* As seen from the table given above, the difference between *B. grandiflora* and *B. Viguieri* is essentially the sum of the differences of each from *B. bursa-pastoris*, so that we can not logically assume that they are specifically distinct from the latter species and only subspecifically distinct from each other.

The non-validity of the sterility criterion is already fully recognized by every one in the case of the Orchidaceae, where fully fertile hybrids are produced, not only between very distinct species, but also between many different genera.

Still another case in Bursa may be cited to show that two forms recognized as species by taxonomists may differ in only a relatively simple genotypic feature which does not affect capacity for interbreeding. *Bursa Heegeri* (Solms-Laubach), which was found by Professor Heeger on the market-place at Landau, Germany, in 1890, is so distinct from *B. bursa-*

pastoris that Solms-Laubach, to whom it was sent for identification, was inclined to place it in the genus Camelina, because of its rounded, uninflated capsules. Only when he observed a mutation (*Bursa Solmsiana* Shull *ined.*) arising from it, which exhibited a partial reversion to the *bursa-pastoris* type of capsule, did he recognize the identity of the new form as a species of Bursa.

I have made a very large number of hybridizations between *B. Heegeri* and different biotypes of *B. bursa-pastoris*, and these two species have bred together with perfect readiness and with full fertility, so long as the biotypes of *bursa-pastoris* used were of European or east-American origin. The segregation showed that a simple Mendelian difference exists between these two species, though this difference is quite generally duplicated through the presence of the corresponding factor or gene in two different chromosome pairs. It is thus seen that genotypically the species-character which differentiates *B. Heegeri* from *B. bursa-pastoris* is on an exact par with the character of leaf-lobing which differentiates the several rosette types of Bursa into those which have the sinuses extending to the midrib (*heteris* and *rhomboidea*) and those which have the sinuses less deep (*tenuis* and *simplex*), which differentials, as we have already seen, can not be used as satisfactory characters in taxonomy because they are too easily suppressed by environmental factors. It is obvious, therefore, that the capsule form is here a satisfactory specific character because it is unmodified by variations in the environment, and because it is therefore always available for use in determining the identity of any individual, while the *genotypically equal* differences in leaf-lobing are not suitable specific characters because they lack this quality of being invariably expressed when the genotypic constellation requisite for their expression is present.

Since there is no natural hiatus in the range of visibility of different phenotypic characters possessed by different individuals or groups of plants and animals, nor in the degree of their persistence under the normal variations of the environment, I believe that my statement, made above, is substantiated, that *species are only quasi-natural entities* and that they are made so by the lack of agreement between external appearance and internal constitution and by the low visibility of many hereditary characteristics. Natural groups there certainly are, but these are the biotypes of the geneticist, not the species of the taxonomist. Only here and there is there a coincidence between biotype and species.

Since the usefulness of the species concept rests upon the exchange value of specific names in scientific and intellectual intercourse, it must be borne in mind that an undue increase in the number of species has the same effect on the exchange value of the species in the intellectual markets of the world that analogous inflation of financial currency has upon the value of any monetary unit involved in such inflation. Compare, for example, the usefulness of Crataegus species today with the decline in value of the

franc and the lira, if not perhaps with that of the mark and the ruble, and let the taxonomist take warning not to destroy the usefulness of the species concept to biology in general by lowering the degree of visibility of the characteristics which delimit adjacent categories. For the minor groups which his more intensive studies may bring to light let him adopt a special terminology adequate to meet his needs in communicating with other taxonomic specialists, just as the geneticists have done. It is to be hoped, however, that the taxonomist will not find it necessary to propose such a profusion of names for these subspecific categories as have the geneticists. Witness: "elementary species," "biotype," "Jordanon," "isoreagent," "genospecies," "microspecies," "microgene," etc.

If all biological specialists, in whatever direction their specialties may lie, should adopt this method, species will continue to represent the relatively crude, relatively superficial triangulation of the entire field of biology by means solely of such instrumentation as is available, in common, to the devotees of every branch of the science, and the specific names will retain their high value as media of scientific exchange.

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THE SPECIES CONCEPT FROM THE POINT OF VIEW OF A MORPHOLOGIST[1]

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On the reality of organic evolution we all agree; but has the species concept outlived its usefulness? It served prior to Darwin as an expression of the dogma of special creation. Does it serve equally well now to represent concrete realities as seen from the evolutionary standpoint? Morphology is essentially a comparative science dealing with phylogenies, and the limiting of this paper to the twenty minutes allowed will permit only of a *résumé* rather than of the presentation of the detailed data as to the true significance of the species concept as related to phylogeny and evolution. On the matter of species it is to be noted that Darwinian evolution dealt rather with the question of origins than with that of content. In this particular the change is not *per se* so basic as we sometimes think. Linnaeus' idea of a species as a recognizably distinct group of interbreeding individuals all having a common descent is still the basis of most of our clear thinking in matters of classification. The evolutionary dictum that species originate as variants from a parent group and are themselves the potential parents of groups yet to be produced does not necessarily affect the question as to their make-up or delimitations at any given time.

Does the concept *species* represent as adequately the unit of evolutionary progress as it did the unit for theories of special creation? The value of a scientific concept is not infrequently in direct proportion to its plasticity as shown in its ability to undergo more or less fundamental and far-reaching changes in its significance with the increase of our knowledge of the subject data on which it is based. For example, in cytology the concept *cell* has shown its utility by surviving such fundamental modifications in content as are involved in the change from its original use as relating to a cavity or box-like chamber in plant tissue to its present use to designate a one- or several-nucleated mass of protoplasm. The changes now going on in the conceptions back of the term *atom* are another illustration in point.

I suppose there never was a time before in the history of science when a theory played such a dominant rôle as has the theory of evolution since the time of Darwin. Never has a viewpoint proved so fruitful of new interpretations, so stimulative of productive research. Its basic concepts dominate alike morphology, physiology, sociology, and such less sharply defined realms as psychology and philosophy. The only comparable case

[1] Read in the symposium on "The Utility of the Species Concept," at the joint meeting of Section G of the American Association for the Advancement of Science, the American Phytopathological Society, and the Botanical Society of America, at Toronto, December 28, 1921.

is that of the atomic theory in the physical sciences, and here it is interesting to note that the modern alchemists with their isotopes and electronic dissociations are threatening to make chemistry and physics evolutionary sciences; at least, they are striving to lay the foundations for a true evolutionary theory of the elements. All schemes of classification aim to be phylogenetic. Their rubrics are based without question on evolutionary concepts and express the knowledge of our time as to genetic relationships between the units classified.

There is no real question that the species concept as used by Linnaeus, so far as it relates to content at any one time, does fit in with the facts of evolutionary phylogeny as we know them. Modern research has made us more certain than ever that the Linnaean species do by and large constitute recognizable groups of more or less freely interbreeding individuals whose interwoven pedigrees constitute a specific fabric less diversified than that of the genus, family, or order. Only extremists deny the possibility of segregating and recognizing such genetic units. Only those who hold that all organisms constitute a web of life or vital fabric of descent in which each individual pedigree is inextricably woven up with other pedigrees so that its identity is lost in that of the whole, or, on the other hand, those who hold that the individual pedigrees are the only recognizable and differentiable units and that these are all of essentially the same order of magnitude, have really cut loose from the use of the species concept.

The question is today not whether Linnaean species are in general good phylogenetic units, but whether, with the discovery and recognition of such divisions of these Linnaean species as our small species, or subspecies, which also constitute recognizably differentiated groups of interbreeding individuals, we should now transfer the term *species* to these phylogenetic units of a lesser order of magnitude, leaving the Linnaean units unrecognized, or perhaps—as has sometimes been hinted by the "splitters" might be desirable—giving them in many cases generic rank. It is of interest to note that such proposals do no violence to evolutionary concepts. Agreement here is practically universal among all ranks of biological students. The question turns largely on what is practical. On this point Hall has put the evidence very clearly. It is indispensable in our systems of classification that we recognize and delimit units of both these orders of magnitude. The geneticists and the plant geographers can give clear expression to their results only by recognizing these lesser units—twigs on the evolutionary tree. It is in them that the variants of genetic modification and many times of geographic distribution are displayed. They, or still lesser races, varieties, etc., are the real material of evolutionary advance. Their recognition in the field and their production in the breeder's experimental plots constitute the two great current means of advance in evolutionary science. On the other hand, the recognition and critical delimitation of the Linnaean units is no less important for the geneticist and plant geogra-

pher in fixing the trends of evolutionary science and of plant and animal migration, as well as in the more popular fields of floristics and elementary botanical instruction.

There is little doubt that, if the categories represented by the Linnaean species were to be dropped and the term *species* applied exclusively to the smaller units, we should find ourselves resorting to the use of such rubrics as *species-groups*, *species-complexes*, etc.

We are passing, in my opinion, at present from a stage of knowledge in which the difficulty seemed to be to discover how specific delimitations could ever be broken down to a stage of knowledge in which our difficulty seems rather to be to find adequate evidence of delimitations between the hitherto recognized specific groups. Intensive breeding experiments and intensive study of geographic distribution have both contributed to this result. Far be it from me to enter the lists for either Mendelism or mutation in the great Oenothera controversy. But we must all agree that the mass of mutants or segregants, one or both, which have been so painstakingly described and pedigreed are well calculated to make the lay botanist, be he morphologist or physiologist, more cautious about affixing a species name to any chance evening primrose he happens to find by the wayside or in a garden. The recognition of the so-called species of Oenothera has become a matter of highly expert judgment and discrimination. In this we have certainly traveled far from the viewpoint of the immediately post-Darwinian biologists, who, while they were convinced that species come about by evolution, were quite willing to despair of ever being able to see evidence of the process going on, much less to initiate and control its course and to begin to trace out the mechanism of variation. We are confronted with a mass of evidence as to species in the making among wild plants, and we no longer hesitate to recognize that the breeder can produce, and has from the beginning of agricultural science produced, modifications of type quite comparable to those which characterize evolutionary species.

It goes without saying, of course, that from this evolutionary standpoint the physiological or biological species which are so common and so sharply marked among the parasitic fungi, and the bacterial races differentiated on the basis of their specific pathogenicities or cultural characters, are just as much to be recognized as evolutionary units as are groups differentiated by structural characters. The description and characterization of such groups is not easy, but their existence as biologic entities can not be questioned. The importance from a practical standpoint of their diagnostic characters makes sure that their careful and adequate classification will always be an attractive field of biological research.

The consideration of such groups as these leads naturally to the question as to the practicability in general of such an evolutionary system of classification as I have been arguing for. Will it ever be possible, in any considerable number of cases at least, to trace out the devious paths by

which the groups of plants and animals as we find them today have descended from those of prehistoric or geologic times? Will it ever be possible even to agree upon the delimitations of the groups as they exist today? Will not generic delimitations always be, as is commonly said, matters of opinion? Even if this be the whole story, be it noted that in the long run we may hope to discriminate between the opinions of the biological genius and those of the biological dullard.

The currency of this statement about generic limitations indicates not only the difficulty of such discriminations, but from one angle their evolutionary unimportance. So far as we know at present, it is a relatively unimportant matter how large or how much subdivided a branch of the evolutionary tree is taken for the genus unit, providing only you do not put with its twigs those from other branches. But, it may be asked, will it ever be possible to get the geneticists to agree on how many these are and how to delimit the mutants or segregants of the Oenothera species? We may best leave this specific question to the future, but that there is coming to be a fair degree of agreement as to the identity and delimitation of many Linnaean species will hardly be questioned. We are demonstrating in some cases that certain groups long suspected of free interspecific hybridization are really guilty. The difficulties of the systematists with such groups are more than compensated by the joys of the geneticists and cytologists with each such new discovery. In fact, the cytologists are even now coming to the rescue in the case of the roses, the Jamestown weeds, and the cereal grains, not to mention minor cases, with a brand new set of earmarks for the identification, characterization, and classification of the systematically difficult members of these groups. But discussion of the proper treatment in an evolutionary system of classification of hybrids and their progeny would certainly carry me beyond the limits of my allotted twenty minutes. In my opinion the systematists have been too much in haste to tie up their specific names with so-called historic type specimens. It is not impossible that we may be able to recognize and characterize in its description the actual biologic type form for a species. As you may be aware, my studies on the morphogenesis of such simple forms as the Pediastrums have led me to believe that specific types are very definite and concrete entities capable of being recognized and adequately described both quantitatively and qualitatively. This attempt to recognize and describe what is biologically typical is certainly the aim with present-day systematists who know their material in the field as well as in the herbarium, and there is little reason to doubt that characterizations so based do in general give a picture of the biologic type of the species in its relations to its subspecies, varieties, etc., and to the other related species of the genus.

It seems to me safe to assume that the work of classification will never be finished till the units large and small are brought as nearly as available evidence permits into their evolutionary sequence.

Morphology is commonly said now to be a dead science. Its last gasps have consisted of such tenuous and ill-founded speculations as to have led to general lack of interest and distrust in its conclusions. That there is too much truth in these utterances is to be admitted. Many morphologists have attempted to bridge the gaps in their knowledge with "all too bold guesses and ill-judged hypotheses."

On the other hand, it is hardly to be imagined that biologists will ever lose interest in the great problems of the evolution of plant and animal types. It is equally clear that we shall never rest content until our classifications of plant and animal species present in the fullest degree possible a picture of their evolutionary descent.

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THE SPECIES CONCEPT FROM THE POINT OF VIEW OF A PHYSIOLOGIST AND BACTERIOLOGIST[1]

GUILFORD REED

The demands of bacteriology for the classification of a group of organisms which on account of their minuteness do not lend themselves to conventional structural differentiation has occasioned the development and utilization of different criteria of organic relationship. These criteria, though they have found little favor outside this restricted field, apply equally to the classification of all organisms; and, moreover, from a physiological point of view constitute a more fundamental species concept than one based on structural differentiation. The fundamental basis of these criteria is the conception that organisms differ in the chemical constitution of their protoplasm. The methods of making the determination may be considered indirect, from chemical standards, but they are none the less precise: namely, the methods of immunology.

I

It has long been known that, when an animal receives an injection of certain substances, antigenic substances, antibodies are developed in reaction. These antibodies combine in the body of the immunized animal or *in vitro* with their antigens to produce the various familiar antitoxic, lytic, agglutinating, precipitating, and many other reactions. From the present point of view the significant feature of these reactions is their high degree of specificity: an antibody reacts only with its particular antigen and with no other. The specificity, moreover, is dependent upon the chemical identity of the antigen. Wells and others have shown that when a known chemically pure protein is used as an antigen its antibody reacts only with that chemically pure protein and with no other protein. Such reactions are now finding favor in biological chemistry as a means of identifying proteins which can not be distinguished by the ordinary analytical procedure. We have, then, in these reactions the most delicate known method of detecting chemical differentiation in the complex constituents of protoplasm.

Application of these methods to the differentiation of bacteria now constitutes an extensive and familiar literature. Any detailed consideration is out of place; a single example will be sufficient for the present thesis. If we have a sample of *Bacillus typhosus* immune serum, its agglutinins will react in high dilution with the organisms used for the immunization,

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i.e., B. typhosus. The maximum dilution in which it will act depends upon the degree of immunization. The serum, moreover, will not react in these high dilutions even with closely related organisms (as the other members of the typhoid-*coli* group), and will not react at all with organisms outside of this group. If in a particular sample the serum reacts with *B. typhosus* organisms in a dilution of 1–5000, it will react with the closely related *B. paratyphosus* in a dilution of about 1–200, and with *B. coli* in one of about 1–20. If typhoid organisms are added to such a serum in excess and later removed by centrifuging, both the specific typhoid agglutinins and the group agglutinins will be removed with the organisms. Similar treatment with the other bacteria of the related group will serve to remove their small content of agglutinins but will not have any appreciable effect upon the specific agglutinin.

Such results as this are confirmed by other immune reactions, and pathogenic bacteriology is a series of such examples. The recent application of this method to the study of the Pneumococci is the basis of one of the most illuminating chapters in modern medicine.

The significance of these results for the present discussion rests upon the fundamental chemical nature of the immune reaction, as previously noted. On this basis it must be concluded that the typhoid organisms react because of chemical identity with the antigenic substances of the individuals used in the immunization; closely related organisms react to a certain degree, because of chemical similarity, and less closely related forms fail to react because of chemical dissimilarity with the original antigen.

II

Although not utilized in practical taxonomy in other groups, we have ample evidence of the existence of similar distinctions. The extensive studies of Nuttall on the immune reactions of vertebrate blood are most significant. Nuttall found that when an immune serum containing precipitins for human blood serum was mixed with the latter in a high dilution, a definite volume of precipitate was formed; but when mixed with other sera the precipitate was quantitatively less according to the degree of relationship of the animals supplying the sera.

The work of Uhlenhuth, Wells, Osborne, Gohlke, and many others has brought forward an enormous amount of evidence to show that chemical differentiation exists between homologous proteins in both plants and animals.

Reichert and Brown have given us some more direct evidences of chemical specificity. Ten years ago they demonstrated that the haemoglobins of vertebrates could be distinguished by their crystallography. The results of these extensive studies indicate that there is a common structure of the haemoglobin molecule, whatever the source of the haemoglobin; that the crystals of a genus belong to a crystallographic group which represents a

generic type; and that the crystals of each species of a genus may be distinguished from those of another species of the genus. The various crystal forms depend, obviously, upon the chemical properties of the haemoglobins. More recent work of Reichert shows that specific and generic types of starch may be distinguished both by the microscopic structure of the grains and by the chemical reactions of the starch.

Such evidence, when it is all considered, is fragmentary compared with the detail of structural differentiation; but it is ample to warrant the conclusion that organisms differ in the chemical constitution of their protoplasm, and it remains for subsequent analysis to determine the full extent of this chemical characterization.

III

The increase in size of cells or organisms involves that formation of new material, which must be largely, if not entirely, synthesized within the developing cell. In many cases these synthetic reactions proceed with great rapidity, whereas outside the cell such reactions, if they occur, proceed at an exceedingly slow rate. The most generally accepted explanation attributes to enzyms the rapid rate of these synthetic reactions. Moreover, the same enzyms which operate in hydrolytic reactions are evidently equally capable of catalyzing the opposite or synthetic reaction. On this basis, therefore, it will follow that under favorable conditions those substances which a cell is able to digest may also be synthesized by that cell.

One of the most conspicuous characteristics of enzyms is their high degree of specificity. From Pasteur's classical demonstration of the relation of *Penicillium glaucum* to the tartaric acids we now have a long and familiar list of enzyms which react only with their particular chemical compounds and fail to react with other compounds. The distribution of enzyms in living cells, moreover, exhibits many limitations. This is particularly evident among the bacteria, and its utilization constitutes one of the unique features of bacterial classification. In the *coli*-typhoid group, for example, the members exhibit a wide range of activity toward carbohydrates: from species which ferment only certain monosaccharides to organic acids, to species which ferment many monosaccharides and disaccharides to carbon dioxid and water. The division of species of bacteria which liquefy gelatine, split certain nitrogen compounds to indol, and coagulate casein, from species which do not possess these enzym activities is one of the most frequently observed of bacterial processes.

The distribution of these enzyms is, moreover, a constant factor. The observation that an enzym is produced in reaction to the chemical nature of the substratum appears to be true only to a certain degree. An enzym already present in a cell may in some cases be produced in increased amounts by reaction with the substratum, or enzym action may be suppressed by the conditions of the environment; but where an enzym is normally absent

from an organism it cannot be engendered, as much bacteriological experimentation has demonstrated.

It may be concluded, therefore, that, as a cell digests or does not digest the substances with which it becomes associated, it synthesizes the substances for which it has hydrolytic or dehydrolytic enzyms.

R. S. Lillie has recently brought forward a theoretical explanation of this synthetic action which, if it can be supported, is most attractive. The cell proteins he considers exert a type of autosynthetic action, presumably by the dehydrolytic condensation of amino-acids. The energy transformation in certain of these reactions he considers to be too great to be accounted for by enzym action, and he finds the formation of electrical circuits between different regions of the cell to account better for the observed reactions.

Considerations of such phenomena as emphasized by Rettger's work on the growth of various species of bacteria in the same mixture of amino-acids and other simpler nitrogen compounds may serve to visualize specific synthesis. From such a mixture the various species grow and multiply in their characteristic form, which necessitates that they synthesize their characteristic body proteins, and these we know from serology to be chemically distinct.

IV

If species are characterized by the presence of specific chemical substances, species continuity as well as individual development must depend upon the constancy of the specific synthetic reactions. Any deviation from the characteristic reaction in a cell resulting in atypical end products will of necessity alter the chemical specificity; which alteration may be exhibited in atypical activity, pleomorphic form, or death.

Some recent data concerning the origin and nature of pleomorphic bacteria may be interpreted as resulting from modified synthetic activity. We found that the form of the influenza bacillus could be greatly altered by the H-ion concentration of the culture medium. In a medium of sufficient acidity or alkalinity to be near the growth-limiting reaction, a large percentage of the organisms appear in very distinct pleomorphic form, frequently a hundred times larger than the typical. Moreover, these atypical forms entirely resist the agglutinating action of specific serum and therefore lack the antigenic proteins of the normal cells, a deficiency evidently resulting from altered synthesis imposed by the reaction of the environment. Such an effect might be expected from the familiar action of H-ion concentration upon the proteolytic enzyms.

The transmission of chemical specificity through a specialized germ plasm has received no attention from the present point of view. Yet we might interpret Guyer's recent familiar results concerning the inheritance of experimentally induced eye defects as a case in point. Guyer prepared an anticrystalline-lens serum by immunizing fowls with macerated rabbit

lenses. This immune serum injected into rabbits produced no direct reaction; but defects of the crystalline lens appeared in the progeny of both male and female through several generations according to a definite ratio.

The immune body for anatomical reasons may not come in contact with the crystalline lens, so that we appear to have two alternatives. Either the immune body is transmitted, enters the germ as a new factor, and appears in the developing embryo with destructive effects upon the lens; or, in the injected animal the immune body combines with the lens determiner in the germ plasm and effects its destruction or alteration. If this be true, according to the fundamental basis of immunology, the immune body combines with the determiner because of chemical identity between the substance of the determiner and that of the immune body antigen, *i.e.*, the lens. In other words, the development of lens is conditioned by the transmission of chemically similar substance in the germ.

It is most significant that, in so far as chemical specificity has been determined, it is in close agreement with relationship based upon structural features. We may be permitted to conclude, therefore, that the constitution of the protoplasm is the fundamental species characteristic, of which form and structure are manifestations. This perhaps strengthens the frequent claim that physiology regards species too lightly, yet at the same time it provides a rational basis for physiological generalization and physiological specialization. We may generalize when we have to do with nonspecific reactions of protoplasm or with agencies which influence in a similar manner the various specific constituents; we must specialize when we have to do with the specific substances of the protoplasm.

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THE SPECIES CONCEPT FROM THE POINT OF VIEW OF A PLANT PATHOLOGIST[1]

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To the plant pathologist the problem of the species concept is not only of academic interest but also of intense practical importance. While pathologists as well as other botanists love to seek truth for truth's sake, most of them are under obligation to seek the truth for the good it may do in the complex process of preventing the world from starving. The delimitation of species is prerequisite to the experimentation and research of the pathologist. It is essential that the specific purity both of the host plant and of the pathogene be known. If these fundamental facts are not assured, the pathologist is likely to find not truth but error; or, at best, the truth is in danger of being badly garbled. The accurate description of species, then, is of primary importance, but it is not an end in itself, but only the means to an end. However, being the means to an end, the accurate description of pathogenic fungi is of greater practical importance to pathologists than to any other class of botanists.

It is to be hoped that there will be no unduly severe criticism on account of what may seem to some an ultra-utilitarian viewpoint. The fact is that the pathologist must learn the effect of pathogenes on the host plant. In order to do this, he first must know thoroughly both the pathogene and the host plant. He must depend, therefore, on systematic mycology and other systematic botany for the tools of his trade. If the tools be unsuitable for his work, either he must improve them or he must make his own. Therefore it is pertinent to inquire what quality of tools have been furnished him in the past.

To the taxonomist of higher plants, and particularly to the geneticist, the pathologist is indebted for the proper attitude toward the host plants which he uses. The geneticists have given us the pure-line conception for higher plants. Pathologists now are avoiding many serious mistakes by taking the simple but fundamental precaution of using pure-line host material. For example, wheat generally has been considered to be close-pollinated. But Hayes has shown that natural crossing may occur in the field. Supposing, then, experiments are made on the biologic specialization of pathogenic fungi attacking varieties of wheat; what reliance can be placed upon the results when bulk material of wheat seed is used? Some of the plants very likely will be heterozygous for resistance. This may

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lead to the deduction of entirely erroneous conclusions regarding the pathogenicity of the parasite, especially when the experiments are being made by investigators who are a little over-zealous in detecting evidence of inconstancy in pathogenes. Serious mistakes of interpretation often are made on account of the use of supposedly pure, but actually impure, host material. It is not sufficient for the careful investigator of wheat diseases, especially when fundamental relations are being sought, to know which variety he is using, but he also must be sure that he is using a single pure line of that variety.

If it is true that the greatest caution is necessary to assure the use of pure lines of host plants, it certainly is true that still greater precautions must be taken to use "pure lines" of the pathogene, because it usually is more difficult to detect genetic differences in the lower organisms. Does the practice under the present species concept give the pathologist the necessary assurance regarding the specific purity of the pathogene?

It will be agreed, at least by most pathologists, and I believe by many mycologists, that pathologists often have derived but little aid and comfort from published descriptions of pathogenic fungi. Possibly this has been due not so much to the lack of a proper species concept as to the failure properly to apply that concept. Until recently species have been delimited primarily on the basis of morphology. But the pathologist can not be content to know what fungi look like; he must know also what they can do, because that is his primary concern. He is compelled to study fungous behaviorism, and, in so far as morphological descriptions aid him in this study, they are extremely valuable. It probably is superfluous, however, to state that morphology alone no longer can be considered a sufficiently accurate basis for determining the specific purity of many organisms.

It would scarcely be profitable, even if it were possible, to define species. In general, however, as applied to fungi, the concept seems to be based on the general underlying ideas, (1) that all the individuals comprising a species are sufficiently alike morphologically to make it possible to differentiate them from individuals of other species by means of morphological characters, and (2) that the characters are relatively stable through successive generations—"a perennial succession of like individuals," according to Farlow.

The grouping of individuals into species, then, is an attempt to make it possible quickly to recognize closely related plants and to call them by name readily. The morphology of the fungus has been used as a criterion of essential similarity of individuals. And unfortunately it often also has been misused. The careless, premature, and rather reckless naming of new species of fungi has been a tremendous handicap to the pathologist. Everyone is so familiar with the unfortunate consequences of this tendency, now happily disappearing, that it is scarcely necessary to cite specific examples. Too often temporary modifications, which are not inherited at all, have

been used as a basis for the multiplication of one true morphologic species into several imaginary ones. And, on the other hand, several distinct species sometimes have been described as a single species, either on account of faulty technique or on account of the fact that the range of conditions under which the fungi were studied was not sufficiently wide to make possible the detection of differences which become apparent only under certain environmental influences. The range of variability of many species of fungi is very wide. The character of growth, the ability to reproduce, and the morphology of the organism may be influenced profoundly by the amount and kind of food available and by environmental conditions. Klebs, Thom, Coons, Duggar and his associates, and other investigators have demonstrated this conclusively.

The pathologist is vitally interested in knowing the morphology of a pathogenic organism, not only on one host and under one set of physico-chemical conditions, but under all possible conditions. And he is especially concerned with the question as to whether essential morphologic identity means also essential physiologic identity. In fact he knows that in many species it does not.

Every one now knows that there may be physiologic races within a morphologic species, and there has been a growing tendency, therefore, to use physiologic characters for the delimitation of species. The bacteria in general are separated into species on the basis of their physiological reactions, and very little objection is raised. There also is a tendency to use physiologic characters more and more in systematic work on fungi. The taxonomic work of Appel and Wollenweber, and of Sherbakoff on Fusarium, and of Thom on Penicillium was based not only on morphologic, but also on cultural or physiologic characters. More and more the description of species is being based on material grown on standard media or on several hosts, and under known conditions. Unless this is done, descriptions often mean nothing, because the so-called species may contain not only several morphologic, but also several physiologic, races.

There seem to be different degrees of specialization into biologic forms, specialized races, chemical species, *Gewohnheitsrassen*, physiologic races, or whatever one chooses to call them. These terms were not all used exactly synonymously originally, but, since the differences represented by the terms seem to be in degree rather than in kind, they are all called biologic forms in this paper. These forms supposedly are practically indistinguishable morphologically, although slight differences are known to occur; but they differ decidedly from each other in their physiologic action. There would seem to be several classes of such forms, although the categories into which they can be placed may represent no really fundamental differences.

Dox has shown that there may be such distinct chemical differences between species of Penicillium and Aspergillus that the species can be recognized more easily by chemical than by morphologic characteristics.

Among pathogenic fungi similar chemical differences apparently exist; there appear to be distinct forms of *Sclerotinia* sp., causing brown rot of stone fruits in the United States, which consistently produce strikingly different types of growth on various synthetic media. The differences between biologic forms of Erysiphe, of *Puccinia graminis*, of *Puccinia coronata*, and other pathogenic fungi, no doubt also are chemical, although the exact nature of these differences has not yet been ascertained. It is significant, however, that the reaction of several biologic forms of *P. graminis "tritici"* to hydrogen-ion concentration differs perceptibly. Whether the physiologic differences between biologic forms can be detected on artificial culture media or only by the action of the forms on host plants, it would seem that the nature of the difference is essentially the same—that is, physico-chemical.

Why were biologic forms not called species when first discovered? Probably because the morphological concept of species had become so firmly fixed that it was considered too heterodox to use physiologic differences as a sole basis for classification. Furthermore, morphologic differences were considered more permanent than physiologic differences. But, even as early as 1898, Farlow read the following to the Botanical Section of the American Association:

> When therefore the botanist denies that physiological species are properly species, he is practically admitting that his own definition, the perennial succession of like individuals, is used by him in a special sense, and he does not seem to be aware that species as he limits them are artificial and not natural. The belief that species should be based on morphological rather than physiological characters rests on the assumption that the former are more likely to be inherited and thus show the temporary attempts of the organism to adapt itself to the environment. It is perhaps a question whether the grounds for this belief are as valid as has been supposed. We readily see the morphological characters which have been inherited, but it is usually only by accident or experiment that we recognize the physiological or pathological qualities.

Biologic forms long were considered to be unstable. Ward, Salmon, Freeman, Freeman and Johnson, Pole-Evans, and Johnson all obtained evidence which led them to conclude that the parasitic capabilities of biologic forms of *Puccinia dispersa*, *Erysiphe graminis*, *Puccinia graminis*, and *Puccinia phleipratensis* easily could be changed by bridging hosts and by other influences. The results of these investigations indicated that biologic forms readily acquire the ability to parasitize normally immune hosts, provided they are grown first on some closely, or sometimes even distantly related, susceptible species of host plant. Thus *P. graminis tritici* is incapable of attacking oats, but can grow on barley. On barley, according to Freeman and Johnson, the rust acquires the ability to attack oats slightly, presumably on account of some chemical change in the biologic form. If biologic forms could be changed so easily, the objection to using their physiologic characters in classification certainly would be valid. But do they change easily?

The so-called plasticity of biologic forms of *Puccinia graminis* has been

investigated thoroughly. For ten years the writer and various colleagues have tried in every conceivable way to change the hereditary parasitic capabilities of *P. graminis tritici* and *P. graminis secalis*. Considerable work also was done with *P. graminis phleipratensis*, *P. graminis avenae*, *P. graminis agrostis*, and *P. graminis tritici-compacti*. It was impossible to induce hereditary changes, or, indeed, any fundamental changes, although the growth of these fungi, like that of other plants, is influenced by environmental conditions. These biologic forms were as constant genetically as were the species of wild and cereal grasses upon which they were cultured. There was no evidence whatever that the inheritance of physiologic characters by these biologic forms depends any less upon real germinal specificity than does the inheritance of structural characters in morphologic species. Reed states that "in studying the races of *Erysiphe graminis* one also gets a strong impression of their constancy and definiteness and they seem as real as though separable by structural features." Dox concluded that species of Penicillium and Aspergillus could not acquire new ability to produce enzyms by any special methods of nutrition; and Brierley was unable to "educate" *Botrytis cinerea* unless the initial culture consisted of a mixed population, although a form with colorless sclerotia did suddenly appear from a single-spore strain. This phenomenon, however, can be explained on the basis of known principles of genetics. Brierley points out clearly that it is quite essential to use pure lines of the organism in "fungus-educability" studies. This point can not be emphasized too strongly. Any one is likely to obtain very striking evidence of rapid changes of biologic forms unless his supposed biologic form itself is pure. For example, until a few years ago it was supposed that the *tritici* form of *P. graminis* could change readily. But the so-called *P. graminis tritici* itself consists of at least thirty-seven biologic forms which can be distinguished from each other readily by their action on certain pure-line varieties of various species of *Triticum*. All of these forms develop normally on various pure lines of *Triticum compactum* and apparently also on several wild grasses. It would be strange, in using such mixed cultures, if changes were not observed. Those hosts which were attacked by several of these forms naturally would appear to act as bridges to the normally immune forms. The longer one works with these forms, the deeper becomes the conviction that they represent as real, as constant, and as genetically pure entities as do morphological species.

But many biologic forms differ from each other not only physiologically but morphologically as well. The forms of *P. graminis* which are separable on the basis of their action on different genera of host plants (in the United States) can be recognized by the size, shape, and color of the urediniospores and also by the size of teliospores and aeciospores, provided these spores are developed on hosts of the same approximate degree of susceptibility and in approximately identical environmental conditions. The differences

between some forms may be only two or three microns, but these differences easily can be recognized by quantitative methods; and they are as constant as are the differences between many recognized species of fungi. There is a consistent average difference of ten microns between the length of the urediniospores of the *tritici* form and that of urediniospores of the *agrostis* form. But even if there were no morphologic differences, these biologic forms are distinct and constant pathogenically, and we must recognize their existence.

In a recent paper Brierley expresses views similar to those expressed in this paper and makes the concrete suggestion that Lotsy's terminology, proposed for the phanerogams, be modified to meet mycological needs as follows. He suggested that the term *linneon* replace the species in the Linnaean sense—the description being based on morphological grounds only; *jordanon* would be based on morphological characters which were demonstrated to be transmissible; and *species* would be established only on the basis of morphologic and physiologic reaction under standardized conditions. The term *modification* would be used to designate non-transmissible effects of external conditions. Whether or not this terminology is adopted, the principles involved are worthy of serious consideration.

The physiological concept already has been added to a certain extent to the morphologic concept of species. We raise no particular objection to basing the determination of a species of the Uredinales partly on life history, and all pathologists use physiologic characters in establishing species of phytopathogenic bacteria. If everything which the pathologist must know is classified, it will be necessary to add more and more of the physiologic concept. The simple fact is that as scientists we ought to want to classify plants on the basis of those characters which are really characteristic, whether they be morphologic or pathologic, and as practical pathologists we must do so. We shall no doubt encounter difficulties, but, as technic becomes more standardized and refined, it will become possible to recognize still less obvious differences in species of pathogenic fungi than we now do.

If the criticism be made that the proposed recognition of physiologic characters in classification would be drawing too fine distinctions, all that can be said is that the real distinctions were drawn by Nature; and, if we are dealing with pathogenic fungi in a practical way, we must recognize these distinctions; and, if we are seeking the ultimate truth regarding fungi, surely we ought to accept it in plant behavior as well as in plant structure.

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THE RELATION OF THE ENZYM PECTINASE TO INFECTION OF SWEET POTATOES BY RHIZOPUS

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It has been demonstrated that, if the proper conditions are maintained in the storage house, sweet potatoes can be kept for a considerable length of time with practically no loss from soft rot. If, on the other hand, the conditions are unfavorable, infection followed by decay may take place.

The cause of the soft rot has long been suspected to be due principally to *Rhizopus nigricans* Ehrb. (14, 19). Before positive proof of its parasitism was obtained, some difficulty was experienced in isolating the organism from the rotting potato and still more in obtaining infection by it. If, as is customary in pathological technique, the plate plantings were made from tissue close to the healthy zone, a sterile culture was almost invariably obtained. If, on the other hand, the plantings were made from rotted tissue several millimeters back of the undecayed tissue, a pure culture of the organism was usually obtained. This suggested that there was some "action in advance" of the growing hyphae. Harter and Weimer (17) showed that this action or dissolution of the middle lamellae in advance of the growing hyphae is due to a substance of the nature of an enzym, which, following the precedents established by Bourquelot and Heressey (1), Jones (22), Euler (11), Zeller (37), and others, they have designated as pectinase.

Bruschi (6) found that the cells of plums on which *Monilia cinerea* (Bon.) Schroter had grown for two days were separated along the line of the middle lamella, and concluded from her results that the enzym pectinase was produced by the fungus. Munn (28) obtained similar results with the fungus causing the neck rot of onions. He demonstrated the production of oxalic acid, but from the nature of its action came to the conclusion that it has little or nothing to do with the maceration of the tissue. Munn clearly demonstrated that an enzym which he calls pectinase was responsible for the disintegration of the tissue noted.

Although *Rhizopus nigricans* has been quite generally accepted as the cause of the soft rot of sweet potatoes, its causal relation has been somewhat difficult to prove. The usual method of placing spores or spores and mycelia upon the unbroken surface or upon a wound usually gave negative results, even when the potatoes were placed in a moist chamber lined with wet filter paper. No better results were obtained when the spores and mycelia were injected deeply into a wound made with a needle or forceps. If, however, the method described by Harter, Weimer, and Adams (19), in which the fungus was grown for one or two days in sweet-potato decoction

and then poured into a fresh "well" made into the potato, was followed, positive results were usually obtained. These results suggested strongly that an enzym was secreted which immediately began to macerate the host tissues in advance of the growth of the fungus. That dissolution of the middle lamellae takes place in advance of the mycelium is further borne out by the fact that a sterile zone of uncertain width is always present between the healthy tissue and the tips of the hyphae. In view of the fact, first, that an enzym seems to play such an important rôle in the decay of sweet potatoes after infection once takes place, and, second, that the original infection is difficult to obtain without first growing the fungus for one or two days on a nutrient medium in which the enzym is secreted, the writers proposed to make a detailed study of the part played by the enzym pectinase in infection.

It should be pointed out here that, while the experiments dealing with this phase of the subject were made with either *R. nigricans* Ehrb. or *R. tritici* Saito, several other species also were found capable of decaying sweet potatoes (20) as well as a number of other vegetables and some fruits. All the different species were found to secrete pectinase (18) and to bring about a maceration of the host tissue in a similar way to that caused by *R. tritici* and *R. nigricans*. It is therefore believed that the rôle played by the enzym secreted by these two species is typical of that played by the other species of the genus.

Pathological Histology

Sweet potatoes decayed by Rhizopus are at first rendered very soft and stringy, water often dripping out of the potato when the skin is broken open. At the outset the color of the tissue is not changed, but later it turns a cinnamon to chocolate brown. On the escape of moisture the cells collapse, the potato dries up, and the whole mass finally becomes hard and mummified. Observed in this stage, it is often classed as dry rot.

That the tissue is killed in advance of the fungous threads has been demonstrated by different methods. Attention has been called to the fact that plate plantings made from decayed tissue adjacent to healthy tissue were usually sterile. "Action in advance" was likewise demonstrated by a microscopic examination of stained sections made through the healthy and the adjacent decayed tissue in which the fungous threads were colored blue by Pianese's stain. The results showed that maceration of the tissue had taken place several cells beyond the most advanced hyphae. Furthermore, similar sections stained in methylene blue demonstrated the presence of the middle lamella connecting the cell walls of healthy tissue, while in the decayed tissue it had completely disappeared.

If bits of decayed tissue floating in water are examined microscopically, the cells, although themselves remaining intact, will be seen to be separated from each other along the plane of the middle lamella. So far as the

writers' observations go, the hyphae pass between the cells but do not penetrate, at least during the earlier stages of decay.

Investigations by the writers have demonstrated that neither *Rhizopus nigricans* nor *R. tritici* produces cytase, which may account for the fact that they do not penetrate the cell wall. The absence of a cellulose-dissolving enzym was demonstrated in several ways. Some preliminary and somewhat crude experiments were conducted in which it was attempted to grow Rhizopus on a nutrient solution with Whatman chemically prepared filter paper as the only source of carbon. A modification of Czapek's nutrient solution in which ammonium sulphate was substituted for sodium sulphate served as a medium. Rhizopus makes a rapid and profuse growth on this medium with glucose or even starch paste as a source of carbon. When the filter paper was substituted for glucose, the fungus made practically no growth. The filter paper appeared to be unaltered, and no reducing sugars could be detected according to the method of Clark (8). On the other hand, when starch paste, for example, is used as a source of carbon, reducing sugars are formed in advance of the needs of the fungus (18).

A second series of experiments were carried out in which the action of Rhizopus on cellulose was studied. A cellulose agar was prepared according to the method of McBeth and Scales (26) from a good grade of filter paper as prepared by Scales (31). A uniform distribution of the flocculent cellulose imparted a milky appearance to the agar. Kellerman (25) showed that if this kind of agar in test tubes is inoculated on the surface with *Penicillium pinophilum* Hedg., the enzym cytase is excreted which eventually clears the medium by the dissolution of the cellulose. Furthermore, he found that, if discs of the clarified agar were transferred sterile to cellulose agar in plates, the cellulose in the latter would likewise be dissolved, thus proving conclusively that cytase was produced. The writers duplicated the experiment of Kellerman with *Rhizopus nigricans* and *R. tritici* but no dissolution of the cellulose took place, which would seem to indicate that the enzym is not produced.

It was suspected that perhaps in the absence of available carbohydrates these organisms were unable to make sufficient growth to produce the enzym, and that, if they were cultivated on a medium on which they would grow independently of the cellulose, they might digest the cellulose. With this possibility in mind the cellulose was added to beef agar and the same test as before was applied, with negative results. The results of these experiments seem to prove the absence of cytase production in the species *nigricans* and *tritici*.

Mode of Infection

The results of different investigators have shown that fungi may enter the host tissue, first, by way of the stomata; second, by penetrating mechanically the unbroken epidermis; third, through both the stomata and the epidermal cells; fourth, by means of an enzym secreted by the fungus which

dissolves the epidermal cells, thereby permitting the germ tube to enter; and fifth, by means of wounds. Blackman and Welsford (2) found that the germ tubes of spores of *Botrytis cinerea* Pers. in turnip juice on the leaves of *Vicia faba* L. penetrated mechanically the unbroken epidermis. Initial infections were never found to occur through the stomata. However, after the penetration of germ tubes through the epidermis had reduced their resistance to infection, the hyphae were seen to enter through the stomata. Dey (10) and Weimer (35) showed that *Colletotrichum lindemuthianum* (Sacc. and Wagn.) Scribner and the basidiospores of *Gymnosporangium Juniperi-virginiani* Schwein. penetrated mechanically the unbroken epidermis of the bean and of the leaves of healthy apples respectively. From the results of these investigations there appears to be little doubt that certain fungi have the power to penetrate the epidermis mechanically independently of an apparent enzymic action. Other fungi have been found to gain an entrance through the stomata. Jones, Giddings, and Lutman (23) picture the entrance into the leaf of the potato by *Phytophthora infestans* (Mont.) De Bary both by way of the stomata and by the penetration of the epidermis. Harter (15) observed the germ tube of *Diaporthe phaseolorum* (C. & E.) Sacc. entering the leaf of *Phaseolus lunatus* L. only by means of the stomata, and Gardner and Kendrick (12) found that *Bacterium exitiosum* Gardner & Kendrick entered the leaf through the stomata, although entrance of the fruit was accomplished only through a wound.

That wounding plays an important rôle in the infection of many crops by various fungi is well recognized. There are in fact organisms often regarded as weak parasites which can infect only through a wound. Even those organisms which are able to penetrate the epidermis or those which usually enter through the stomata frequently gain an entrance to the host through a wound. Hurd (21) found that an unbroken seed coat of wheat or barley ordinarily affords absolute protection against attacks on living seeds by Penicillium or Rhizopus in damp storage, in the soil, or in blotter germinators. Infection of such seeds was obtained, however, by retarding germination of the seed by means of low temperatures. Orton (30) found that infection of the Irish potato by *Rhizopus nigricans* was accomplished only through some abrasion in the skin.

The part played by enzyms in infection of the host has been a subject of study and investigation for a long time, and some of the earlier investigators noted what they believed to be cases of the entrance of a fungus only after the epidermis had been softened or weakened by means of an enzym. Busgen (7) and Ward (34) believed that the germ tube of *Botrytis cinerea* accomplishes its entrance into the tissue of the host with the aid of an enzym which dissolves the epidermal walls of the host. Voges (33) and Miyoshi (27), working with Fusicladium and *Botrytis cinerea* respectively, are of the opinion that softening of the cuticle by an enzym or some

dissolving agency precedes the penetration of the cells. Miyoshi was able to show that *B. cinerea* could penetrate mechanically a membrane such as gold leaf. The work of De Bary (9), Nordhausen (29), Smith (32), and others has been referred to in this connection in other papers and will be reviewed only briefly here. De Bary noted that the expressed juice of certain plant organs which had been decayed by *Sclerotinia libertiana* Fuckel if heated was no longer active, and therefore concluded that the cell-wall-dissolving action is due to an enzym. A toxic action was also noted, but to just what this action is due he was uncertain. Nordhausen as well as Smith claimed the production of a toxic substance which penetrates the cuticle and kills the underlying cells.

Some interesting results in this connection are those of Brooks (3), who attempted to infect lettuce by placing spores of *Botrytis cinerea* from a grape-extract medium, dry and in drops of water, on the leaves of plants kept uncovered in a greenhouse. Although some of the spores germinated, no infection was obtained, even when the plants were confined under a bell jar. If, however, young mycelia were placed in drops of the grape-extract medium upon healthy leaves, infection took place. Wounding insured infection. He says:

> In such cases the juices which exude from the wounded areas provide saprophytic nourishment for the further development of the germ tube. Infection could be brought about on leaves which had only just begun to turn yellow, but not on normal green leaves. Botrytis can not be considered a true parasite since it kills tissue in advance of the growth.

The results of the investigations cited above show that some organisms can infect although there is no apparent injury of the outer layer of cells of the host, while others require a wound. That Rhizopus belongs to the latter class has been demonstrated repeatedly in the following manner. Sweet potatoes of the Yellow Jersey variety, one of the most susceptible varieties, which had been cured in the usual way in the storage house, were used in these experiments. Sound potatoes were carefully washed, and a glass ring about one centimeter deep and one and one half centimeters in diameter was sealed on an uninjured spot on the surface by means of a wax made of beeswax and vaseline. A 48-hour-old culture of *Rhizopus tritici* grown in about 2 cc. of sweet-potato decoction was poured into the glass cell, which was then covered with a cover slip held in place by vaseline. In no case did infection take place. This seems to prove that this fungus is unable either to penetrate mechanically the unwounded skin of the potato or to secrete an enzym which will dissolve it.

A mature sweet potato has no true epidermis. Instead of an epidermis, which probably sloughed off early in the growth of the potato, there is a layer of cork two to four cells deep. This layer, which will be designated hereafter as the skin, is able to withstand the attack of the fungus itself or digestion by any of its secretions. A sweet potato inoculated with *Rhizopus tritici* will entirely decay with the exception of the skin, which the hyphae

are unable to penetrate even from within. If, however, some of the outer cells are ruptured, the fungus grows out and forms sporangia on the surface. Likewise, if sections of sweet potatoes cut to include some of the skin are immersed in an extract of the fungous hyphae or in a solution on which the fungus has grown, all the tissues except the cork are macerated and separate readily from it. The skin, therefore, forms an effective barrier to the penetration of Rhizopus.

Small dead rootlets were proved to be the point of entrance of the fungus in a small percentage of the trials made. These experiments were conducted by the use of the method just described. A glass ring was sealed over an old dead rootlet, and a 48-hour-old culture of the organism grown in about 2 cc. of sweet-potato decoction was poured into the cell, which was then covered with a cover slip. Out of a large number of such trials only about 25 percent of the potatoes thus inoculated became infected, while 100 percent of the controls inoculated by the well method decayed. It may be concluded from these results that the rootlets do not form as effective a barrier to the penetration of the fungus as the unbroken skin, although it was shown by Weimer and Harter (36) that a somewhat incomplete cork layer is laid down beneath the rootlets if the proper conditions of temperature and humidity are maintained. It was also shown that if the solution on which the fungus had grown was poured into a glass cell sealed over a dead rootlet, more or less softening of the tissue took place in some cases. In other words, the dead rootlets formed a point of entrance which was not in all cases effectively protected by a cork layer. A number of sweet potatoes from the storage house, which had some wounds made during digging and storage but no apparently fresh ones, after being held under running water to remove some of the dirt, were immersed in a sweet-potato decoction on which the fungus had grown for 48 hours. After about 24 hours in this solution, there was softening at the end where the potato was separated from the stem, in bruises and wounds made during digging and handling, and at certain places where small rootlets had died. These results show that in practically every potato certain wounds are present through which the enzym can enter; *i.e.*, the skin which forms the only barrier to the entrance of the fungus is ruptured.

A study was made of the extent of wounding necessary to permit infection when the fungus was grown for one or two days on sweet-potato decoction and the decoction and mycelium were used as an inoculum. Different types of wounds were tried. When such a growth was poured into a "well" made by means of a cork-borer, infection usually resulted. On the other hand, only about 50 percent of the attempts to inoculate sweet potatoes through a small scratch just sufficient to rupture the skin were successful. When the skin was punctured once with a needle, the percentage of infection was even less (35 percent). These results show that a very small wound is sufficient to permit infection if the enzym is present. No infection

resulted when similar experiments were conducted using spores and hyphae in water and in the decoction instead of the one- and two-day-old cultures. Weimer and Harter (36) showed that cork is formed over wounds when the proper conditions of temperature and humidity are maintained. This wound cork was likewise found to exercise some resistance to infection by Rhizopus, but not complete protection against invasion. Numerous observations of sweet potatoes in storage houses led to the general conclusion that infection there takes place at the ends of the potato more frequently than elsewhere, which would seem to indicate that what wound cork is formed there, together with the latex congealed over the surface, is not a complete protection against infection. Experiments in which 24-hour-old cultures of the fungus grown on sweet-potato decoction were poured into glass cells sealed over the ends of potatoes which had been in storage for some weeks showed that infection could take place through the ends.

The results thus far show that infection takes place only through wounds. Furthermore, it is evident from the results that the wound need not be large, a mere needle prick being sufficient if the enzym is present.

Saprophytic Start

When sweet potatoes are dug, each one is wounded where it is broken from the vine. Furthermore, the skin of the potato is likely to be ruptured to some extent in the ordinary farm operations of digging, handling, and storing. The results of inoculation experiments with *Rhizopus tritici* on sweet potatoes and other crops show that it seldom if ever infects except through a wound of some sort. However, inoculation experiments have conclusively demonstrated that a wound alone is not sufficient to insure infection. Hundreds of attempts to infect sweet potatoes by smearing spores alone, and spores and hyphae dry, on the surface of a fresh wound have for the most part been unsuccessful, even when the potatoes thus inoculated were subjected to the temperature best suited to the growth of the organism and to a relatively high humidity such as that obtained by confining them in a moist chamber with wet filter paper on the bottom.

Furthermore, if water in which spores and hyphae are suspended is poured into a "well" made into the potato by means of a cork-borer and covered with a cover slip to prevent evaporation, consistent infection does not take place. Likewise, if sweet-potato decoction is used instead of water, only a small percentage of infections results, in spite of the fact that the spores in both cases germinate and that those in the sweet-potato decoction form a considerable amount of fungous growth. Sweet potatoes absorb liquids quite readily, especially through a cut surface, and in all cases such as those just described the liquid was absorbed by the end of 24 to 48 hours. Whether or not this is the reason why infection does not take place under the conditions of these experiments is not clear. However, results which throw some light on this question were obtained by experi-

ments in which a strip of cheesecloth with one end in a beaker of water and the other in the wound over the spores kept the spores constantly wet. Germination took place readily under these conditions, but infection did not occur. Likewise, when cut potatoes were immersed in a spore suspension and then confined in a moist chamber, only a very low percentage of infection was obtained. As a matter of fact, the total percentage of decay by the use of this method was no greater than that of the controls which were not inoculated.

The results of the investigation so far show that sweet potatoes are difficult to infect by the usual laboratory methods. On the other hand, potatoes wounded during the winter and kept in a commercial storage house frequently decay. The interesting question in this connection is why it is so difficult to infect sweet potatoes inoculated by the usual laboratory methods while those freshly wounded and held in a storage house decay so readily.

Keen (24) stated in respect to the decay of sweet potatoes caused by *Rhizopus nigricans* that the organism must first have a saprophytic start in order to become a parasite. He found that, if the spores were germinated and allowed to grow for a short time in orange juice, and were then transferred to slices of sweet potatoes, decay would take place. Brooks (3) was likewise unable to infect lettuce with *Botrytis cinerea* by the use of spores alone, but if young mycelia were transferred to drops of grape extract, infection resulted. Neither of these investigators attempted to explain the principle underlying the "saprophytic start," but it would seem that both are cases in which an enzym played an important rôle.

So far as sweet potatoes are concerned, infection probably rarely takes place by the entrance of the hyphae directly into the healthy tissue, either wounded or unwounded. Many experiments, some of which have already been referred to, have shown that, even when potatoes are cut in two and dipped in a suspension of Rhizopus spores, infection, if it occurs at all, begins not on the cut surface but at some point at the edge of the cut where there is a bruise or dead tissue which serves to give the organism a saprophytic start. Such a "saprophytic start" is likewise furnished when the fungus is grown for one or two days in sweet-potato decoction. As already pointed out, when this method is followed, the decoction and fungous growth being confined in a "well" made into the potato, infection is practically assured. That this method is not the only one that enables the fungus to infect is evident from the following experiments. A number of potatoes were cut in two, and the cut surface of one half was held over a Bunsen burner until slightly charred. Treatment of this sort killed the tissue for several cells beneath the surface. All the halves of the sound and burned surfaces were smeared with spores of *Rhizopus tritici*. In 20 hours the fungus was growing on the surfaces of the burned potatoes, and at the end of 2 days these potatoes were about three fourths decayed. The control halves, whose surfaces were not charred, remained sound.

Rhizopus grows readily on almost any kind of culture medium. A synthetic medium made somewhat acid to prevent the growth of bacteria is generally used in isolating the different species from dead or decayed material. Sweet potatoes which have been carefully washed have been dipped into a suspension of Rhizopus spores in synthetic agar. Infection and decay of potatoes so treated have always resulted. In these experiments no fresh wounds were made, infection in every case taking place through old wounds or at points where there were dead rootlets. A sweet potato completely covered with a coating of agar is under an abnormal condition, since respiration is largely cut off. Oxygen starvation is in the end inevitable, and the resistance of the host is doubtless greatly reduced. Other experiments were conducted in which agar with spores suspended in it was placed on a fresh wound. Decay took place as in the former case but not in so short a time, since infection occurred at only a single point. These and other experiments which will not be detailed show that if Rhizopus is given a saprophytic start, either by growing one or two days in a decoction in agar on a wounded surface of the host, or in dead cells on the host, infection will almost invariably take place.

The Relation of Certain Other Factors to Infection

It is obvious that a temperature that will permit of the growth of the fungus must be maintained. The optimum temperature varies with the different species, as shown by Harter, Weimer, and Lauritzen (20). It is generally assumed that relatively high humidity is required for infection, but there is some evidence to show that infection will take place when the humidity of the air is comparatively low. Also, infection frequently fails when the humidity is relatively high. In fact, it is well known that sweet potatoes will become infected in the storage house under what might be regarded as fairly dry conditions. On the other hand, sweet potatoes, after being immersed in a spore suspension, often fail to decay when confined in a moist chamber lined with wet filter paper. Differences in the method of treatment previous to immersion in the spore suspension have given quite opposite results. If, for instance, sweet potatoes are cut in halves with a knife, dipped in a spore suspension, and then confined in a moist chamber, infection usually does not take place. If, on the other hand, the potatoes are struck against a blunt edge so as to make a wound ¼ to ½ inch deep, and are then dipped in a spore suspension, infection almost invariably results. In the former case, the surface of the potato probably dries off by the absorption of the water before the spores can germinate and infect. In the latter case, on the other hand, the mutilated cells and the cell sap form a substratum which retains sufficient moisture to permit the germination of the spores and to provide for the subsequent growth of the mycelium.

Reference has already been made to some experiments in which the

potatoes, after being cut in halves and dipped in a spore suspension, were confined in deep preserving jars. Some of the jars were plugged with cotton, some were left open, and others were closed with a close-fitting glass stopper. No decay took place, either in the jars that were left open or in those that were closed by glass stoppers. In the former case, some hyphae grew on some of the potatoes but no infection resulted. In the latter case, no hyphae were seen, which was probably due to the fact that the carbon dioxid which accumulated in the jar was injurious to the fungus. Saturated filter paper or cotton in the bottom of the jars did not materially increase the percentage of decay. If, however, air is constantly pulled through water and then through the jar, infection takes place. On the other hand, air circulation was unnecessary when the germinated spores and decoction on which they grew were poured into the "well," since it was found that, if the cover glass over the well was sealed on airtight, infection would still take place.

The Part Played by Enzyms

In this connection the writers have in mind pectinase, which they have shown is abundantly produced by Rhizopus, and which they have found is capable of macerating the tissue of sweet potatoes (18). It has been demonstrated that a part of this enzym is exuded from the mycelium into the substrate; also that a watery extract of the mycelium and the enzym exuded into the solution on which the fungus had grown would disintegrate the tissue of thin sweet-potato disks in from 2 to 4 hours. It is likely that pectinase passes into the substrate almost immediately upon the germination of the spores, and it is not unlikely that it may diffuse from the spores even before germination, since it has been shown that a watery extract of the spores will macerate raw sweet-potato tissue[1] in the same identical way as an extract of the hyphae or as the solution on which the fungus has grown. Although the exudation of pectinase into the substrate from living ungerminated spores has not been demonstrated, it has been shown that it is exuded into the solution soon after germination. The following experiment was designed especially to throw some light upon this question. A large volume of spores of *R. tritici* was suspended in sweet-potato decoction in 2-liter Erlenmeyer flasks and incubated at 35° C. After 6 hours, the decoction from one of the flasks was filtered through no. 2 Whatman filter paper to remove the spores and mycelium. Raw sweet-potato disks ½ mm. thick and 1.5 cm. in diameter were partially macerated in the solution in 24 hours. A control of the same solution steamed to inactivate the enzym produced no maceration. Only a part of the spores were germinated, with germ tubes varying from one to several times the diameter of the spore. At this stage it is to be expected that there would be only a minimum amount of pectinase present. The strength of the macerating

[1] Results not yet prepared for publication.

principle would naturally depend upon the number of spores per unit volume of solution.

The second flask was taken off at the end of 7 hours, the solution was filtered, and raw sweet-potato disks from the same source and of the same size were suspended in it. Maceration of the tissue in this case was much more rapid, being quite advanced in 24 hours and complete in 45 hours. The spores were mostly germinated, and the amount of hyphal growth was far more abundant than in the flask removed an hour earlier. A duplicate experiment with *R. nigricans* gave similar results, although the rate of maceration was somewhat slower than in the case of *R. tritici*. It would seem from these results that a substance capable of dissolving the middle lamellae is secreted early in the process of germination, and that this macerating principle very rapidly increases in amount at least in the early period of growth. The maximum is reached in about 2 days in the solution and in about 3 days in the mycelium (17).

It has been shown that this enzym is produced early in the germination of the spores (6 hours). More recent researches (not published) have shown that the spores themselves independently of their germination contain an enzym which, when extracted with water, will bring about the disintegration of raw sweet-potato disks.

The data presented thus far show fairly conclusively that Rhizopus is unable to penetrate mechanically the unbroken skin of the sweet potato. It also shows that, even though the spores and hyphae alone are placed on a fresh wound, infection usually does not take place, although what might be regarded as favorable temperature and humidity are provided. It was, however, shown by a number of experiments that if the fungus is grown for a day or two on a decoction made of sweet potatoes, and this, together with the mycelial growth, is poured into a "well" in the potato, infection will almost always take place. The writers showed elsewhere (17) that if raw sweet-potato blocks were immersed in the substratum on which the fungus had grown, after it had been freed of the fungus, or in a watery extract of the dead mycelium, a rapid dissolution of the middle lamellae took place. This action on the living tissue is in every respect identical with that produced when the fungus itself is decaying the potato. Attention was also called to the fact that in decaying sweet potatoes there is a zone of disintegrated tissue adjacent to the sound tissue which is sterile of the fungus. These and other results show that for this fungus at least a saprophytic start is nearly if not always required before infection takes place. That enzyms play a part in the decay of various plant organs is pretty generally agreed, and that they play an important rôle in infection has been suggested. De Bary, Ward, Nordhausen, Smith, and others noted what undoubtedly was enzymic action. Although the evidence as presented by them does not unqualifiedly prove the action of an enzym, there can be little doubt that they were dealing with what the writers have regarded

as an enzym. Brown (4) has shown that the germ tube of *Botrytis cinerea* is unable to affect chemically the cuticle of the host, nor does it secrete any toxic substance which can pass through the cuticle and bring about the death of the underlying cells. He found that the fungus is unable to affect the underlying tissue until the obstacle offered by the cuticle has been removed. Penetration of the cuticle must take place in a purely mechanical way. In this connection, in another paper (5), he says:

> Once penetration of the cuticle has taken place the problem becomes simply an enzymological one. Further, in the case of the so-called wound parasites the problem presented is much simpler as the problems which arise antecedent to the penetration of the cuticle do not come into consideration.

Gortner (13), in similar studies, found that *Sclerotinia cinerea* when grown on prune- and apple-juice media elaborated a very active pectase. When the fungus penetrates the host tissue it dissolves the middle lamellae, forming a product which, instead of being assimilated as food, is precipitated as a certain compound of calcium pectate. Rhizopus is one of those organisms that are unable to make their way into plant tissue which has not previously been disintegrated by enzymic action. In view of this fact, the fungus evidently depends upon the secretion of the enzym antecedent to infection. When exposed to sufficient moisture and to the proper temperature, the spores germinate. Although the spores may germinate on a fresh wound, they seldom make sufficient growth to infect. The results seem to indicate that infection must start where there are dead cells or tissue on which the fungus can grow. In these dead cells the growth of the hyphae is accompanied by the secretion of the middle-lamella-dissolving enzym. This enzym secreted by the growing mycelium, when once it comes in contact with the healthy host cells beneath, brings about a disintegration of the tissue which is later invaded by the hyphae.

Summary

1. Rhizopus can not infect sweet potatoes through the unbroken skin. Spores and hyphae smeared on a freshly cut surface will produce infection only rarely. However, when the fungus is given a saprophytic start by growing on dead rootlets, in synthetic agar solidified on the cut surface of the potato, or in dead cells killed by charring over a Bunsen burner, infection takes place readily. Furthermore, infection can be brought about readily by growing the organism for one or two days in sweet-potato decoction, if the decoction and mycelium are poured into a "well" made in the potato and then sealed over with a cover glass to prevent evaporation. Infection is accomplished only after the dissolution of the middle lamellae by means of an enzym (pectinase) secreted by the growing hyphae. In practically all cases infection takes place in wounds where there is some dead tissue upon which the fungus can get a saprophytic start. During the growth of the mycelium in these dead cells, the enzym is produced which, when it

comes in contact with the living cells of the host, dissolves the middle lamellae; the cells then die, and a suitable substance for the further development of the fungus is provided.

2. The practical significance of these results is that wounding is a preliminary necessity to infection. Although sweet potatoes are necessarily wounded at digging time when they are broken from the stem, other wounds made by rough handling during harvesting, storing, and preparing for the market should be avoided as much as possible.

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THE INHERITANCE OF FLOWER TYPES AND FERTILITY IN THE STRAWBERRY [1]

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In a study of sterility in the strawberry begun by the writer in 1914, a portion of the work was directed toward determining the underlying factors causing "nubbins" or imperfectly developed berries. These are commonly produced from the tertiary and later flowers of the inflorescence of many cultivated varieties of strawberry and result in considerable loss of fruit toward the close of the picking season.

A study of the fruiting habit of the wild American strawberries and of the cultivated varieties proves conclusively that the production of nubbins is directly related to pistil sterility, and that pistil sterility is decidedly more prevalent on plants with certain flower types than on others (7). Therefore, since the question of fruitfulness in the strawberry is primarily one of sex, a thorough knowledge of the flower types and of their inheritance is essential to the strawberry breeder if his work is to be other than blind crossing and selecting for chance high-yielding clones.

The work on the inheritance of flower types in the strawberry has been discontinued by the writer, but, as some facts have been determined, he presents the data obtained and the conclusions drawn from them.

Flower Types in the Strawberry

In the cultivated strawberry, pistillate and perfect flowers are commonly encountered. The pistillate flowers bear small abortive stamens which have never been observed by the writer to produce pollen. The pistils are generally very fertile, producing perfect fruits from most of the flowers and comparatively few nubbins. The perfect-flowered varieties develop anthers which produce varying amounts of normal pollen. As a class, these varieties are less fertile than the pistillate varieties and produce a higher percentage of nubbins and of sterile or male flowers (7).

The wild species of American strawberries may be divided into two types: those which bear only perfect flowers, as *Fragaria americana*, and those which are dioecious. The pistils of the former species are very fertile, and nubbins or sterile flowers are seldom seen. The dioecious types produce pistillate plants and plants which apparently are hermaphrodites but are in fact staminates. The pistils of the pistillate clones are usually fertile, but the pistils of the staminate clones are rarely so, and the few berries

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developed are practically always irregular nubbins. These clones are somatic hermaphrodites; *i.e.*, they appear to be perfect but the pistils are sterile. Occasionally other flower types are found in *F. virginiana*, such as completely sterile clones in which pollen formation never proceeds farther than the tetrad, but such forms are comparatively rare and need not be considered (7).

Material

The material to be considered in this paper was the result of crosses made at the Minnesota Agricultural Experiment Station in 1915 and 1916. The seedlings were later transferred to the Minnesota State Fruit-Breeding farm at Zumbra Heights. The individual plants were set in the field where they were allowed to multiply for one season. The following season, records were made as to the sex condition and the degree of fertility of the various clones. In nearly all cases the records obtained are the result of observations of the sex condition and fertility of several plants of each clone, and therefore probably represent fairly accurately the sex condition of these clones under field conditions. A few which did not blossom in 1917 were left until 1918 when final records were taken. The writer is indebted to Mr. John Bushnell for completing the records on certain crosses in 1918 while the former was absent in military service.

Terminology

For the sake of convenience and clearness in discussing the crosses of the various flower types, symbols will be used to represent the various sex determiners. Shull (4) suggested in the case of Lychnis the use of the symbol *FF* to represent a female and *FM* to represent a male, as he determined that the females were homozygous for the sex determiners whereas the males were heterozygous. Similar symbols have been found appropriate in interpreting the sex condition in the grape (5). It will be apparent from the following discussion that these symbols will not apply in the case of the strawberry, since the females are apparently heterozygous for the female and male determiners and the males homozygous. Therefore the symbols *FM* and *MM* will be used to designate the genetic condition of the female and male plants respectively of the dioecious wild forms, and *FH* and *HH* the females and hermaphrodites respectively of the cultivated varieties, as it has been shown that the cultivated hermaphrodites have probably been derived from partially fertile wild male types (7). In the strawberry, as in the grape, it may be assumed that the factor for femaleness (*F*) carries linked with it the suppressed male factor, and that the functional male factor is linked with a factor for femaleness which is suppressed to the extent that only somatic structures are developed and the pistils are sterile. In the case of the hermaphroditic strawberries, the female condition is not suppressed, and the pistils are therefore fertile. The symbol *H* then represents the linked factors *F* and *M*.

The seedlings studied fall into five groups based on parentage. These are as follows: Hermaphrodite × self, hermaphrodite × hermaphrodite, male × male, female × hermaphrodite, and female × male. The various combinations will be considered separately.

The term *female* will be used in referring to pistillate plants bearing abortive anthers; *male* in referring to the somatic hermaphrodites which generally bear sterile pistils, and the term *hermaphrodite* in referring to those perfect-flowered forms which are fertile with regard to both pistils and pollen.

Hermaphrodite × Self

Table 1 gives the results of self-pollinating hermaphroditic varieties of strawberry. A total of 352 hermaphrodites, or somatic hermaphrodites, and no pistillates were produced when 11 distinct clones were selfed.

Table 1. *Results of Self-pollinating Perfect-flowered Varieties of Strawberry*

Lot No.	Variety or Seedling Selfed	Hermaphrodites	Sex of Progeny	
			Pistillates	Staminates
1/15	778	68		
1/16	F_1 of (778 × self)	2		
32/16	F_1 of (778 × self)	18		1
57/16	F_1 of (*F. virg.* ♀ × 778)	46		2
6/15	South Dakota	19		
3/16 and 40/16	Dunlap	72		
4/16 and 26/16	Minn. no. 3	59		
63/16	Fendell	3		
	Prolific	2		
54/16	F_1 of (Glenville × self)	5		
15/16	Glenville	28		27
	Total	322		30

In view of the number of different parent plants used, the results go to prove that hermaphrodites do not carry the factor for femaleness and are therefore homozygous for the hermaphrodite determiners. These results are in keeping with the condition found in the hermaphroditic wild species *F. americana*, which must be considered to be homozygous for the hermaphrodite determiners. They are also in keeping with results of strawberry breeders who never expect pistillate varieties to result when only hermaphrodites are used as parents.

Although all these seedlings were hermaphrodites as far as somatic condition is concerned, there were distinct differences in the fertility of some of them. Lot 32/16, an F_1 of 778 × self, produced 18 fertile plants and one which was sterile. Sterility in this one may have been the result of weakness, due to self-pollination or to some other cause.

An hermaphroditic F_1 progeny of *F. virginiana* ♀ × 778 ♀ (lot 57/16)

when selfed produced 46 hermaphrodites of varying degrees of fertility, and 2 males which were completely sterile. As will be shown later, the pistillate strawberry plants appear to be heterozygous for sex, and therefore the female grandparent *F. virginiana* would be of the constitution *FM* and when crossed with 778 (*HH*) should give 1 female (*FH*) to 1 hermaphrodite (*HM*). *HM* was the supposed constitution of the hermaphrodite selfed in lot 57/16. The expectation from it when selfed would be 1 *HH* : 2 *HM* : 1 *MM*. There were produced 46 hermaphrodites and 2 males, one of which was very weak. The hermaphrodites varied in fertility. Five were completely fertile in all flowers; 26 set all but quaternary flowers; 14 were fertile in primary and secondary flowers while the tertiaries were either sterile or produced nubbins and the quaternaries were sterile; 1 produced only nubbins and was practically a male. Although it is difficult to classify each of these with respect to its genetic constitution, it seems apparent that all the expected classes are represented; the *HH* being those completely or nearly completely fertile, the *MM* those completely sterile, and the *HM* group those showing intermediate degrees of fertility. These results are significant of the constitution of the wild female, as they show conclusively that the tendency toward sterility of a dioecious species may be transmitted by the wild female, which is fertile, to hermaphroditic progeny; whereas its female progeny are always completely fertile. A probable explanation for the variations in fertility of the *HM* individuals will be discussed later.

Lot 15/16, Glenville × self, although conforming to expectation as far as somatic characters are concerned, was strikingly different as to fertility from the other selfed hermaphrodites. This variety was mentioned in a previous paper (7) as being practically sterile when grown under field conditions although it produced blossoms profusely. When grown in a bench in the greenhouse it exhibited the same sterility in the first crop of blossoms, but when potted and kept in the house until a second crop of blossoms was produced it exhibited a fairly high degree of fertility. It is evident that environment plays an important part in the fertility of certain clones. This variety (15/16) when selfed produced 55 seedlings, 27 of which were completely sterile under field conditions while 28 produced some fruit. Of these, 11 exhibited a fairly high degree of fertility under field conditions, while the remainder set only an occasional flower and the berries produced were often nubbins. One seedling, which produced an occasional berry when grown in the greenhouse, was self pollinated (54/16). It produced 5 seedlings all of which were sterile to a high degree, setting only an occasional achene on some of the primary flowers. It appears from the results obtained that Glenville carries an hermaphrodite factor which is fertile and another which is practically sterile. Under certain cultural conditions, the first of these factors is dominant and fruit results, while under other conditions the other is dominant and the plant is sterile.

Hermaphrodite × Hermaphrodite

The cross hermaphrodite × hermaphrodite should give the same results as to sex types as hermaphrodite × self. The results of 11 such crosses are given in table 2.

Table 2. *Results of Crossing Perfect-flowered Varieties of Strawberry*

Lot No.	Varieties Crossed	Sex of F_1 Seedlings		
		Hermaphrodite	Pistillate	Staminate
14/16.	Fendell × Dunlap	25		
23/16.	Fendell × Minn. no. 3	4		
71/16.	(F_1 of S. Dakota × self) × (F_1 of 1017 × Progressive)	32		
24/16.	(F_1 of 778 × self) × Minn. no. 3	2		
34/16.	(F_1 of 778 × self) × Glenville	20		
12/16.	Dunlap × Glenville	8		
47/16.	(F_1 of *F. virg.* ♀ × 778) × Glenville	14		
47/16.	(F_1 of *F. virg.* ♀ × 778) × Glenville	4	5	
48/16.	Minn. no. 3 × Glenville	28		
53/16.	(F_1 of 778 × self)* × Glenville	67		
18/16.	(F_1 of Glenville × self) × Minn. no. 3	4		
	Total	208	5	

* Stamens of intermediate type between staminodes and perfect stamens.

These results are similar to those obtained by selfing hermaphrodites, with the exception of one cross which gave apparently a 1 : 1 ratio of hermaphrodites and pistillates. These were the progeny of a cross in which an hermaphroditic F_1 seedling of *F. virginiana* ♀ × 778 ♀ was used as the female parent and Glenville as the male parent. According to our hypothesis, the female parent (hermaphrodite) derived from *F. virginiana* ♀ × 778 should be of the constitution *MH*, the *M* representing the male determiner carried by a wild female. Glenville we may consider to be *HH*. From such a cross all the progeny should be hermaphrodites at least as far as somatic condition is concerned. It seems probable that this was the case in view of the stamen condition found in a portion of the flowers in other combinations in which 778 was used. When 778 was selfed it produced only hermaphrodites. Some of these developed stamens which produced only a small amount of pollen in some flowers while the stamens of other flowers were practically identical with the staminodes of pistillate plants. One of these clones was used as the female parent in a cross with Glenville (table 2, 53/16). This cross produced 67 hermaphrodites. Forty-seven of these developed normal stamens in all the flowers. Twenty showed a decided tendency toward the production of staminodes similar to those found in pistillate flowers or of the intermediate type found in their female (hermaphroditic) parent. Eleven of the twenty bore flowers which pro-

duced many staminodes and intermediate stamens and a few normal ones, while the other 9 produced many flowers which could not be distinguished from those borne on pistillate plants. Only an occasional perfect flower appeared to indicate the true sex condition of the plants. I believe that the five apparently pistillate seedlings resulting from the cross 47/16 were of this type, but were not observed at a time when perfect flowers were present.

Of two hermaphrodite × hermaphrodite crosses made by Mr. Charles Haralson, one, 1017 × Francis, resulted in 43 hermaphrodites, all very fertile, and no females; the other, 1017 × Progressive, resulted in 1,105 hermaphrodites and 33 females. Here, again, the writer believes that these were not true females but were hermaphrodites observed at a time when no functional stamens were present. This view is strengthened by the fact that in this lot of 1,138 seedlings, 433 hermaphrodites produced some female flowers. In some of these the primaries only were pistillate, while in other clones only an occasional perfect flower was produced (7, Pl. 35). An occasional pistillate flower is quite common in many of the hermaphroditic varieties, especially among the primary flowers produced early in the spring. They are always fertile to a high degree, and produce the largest berries borne on the cluster. Wild males of *F. virginiana* may also produce an occasional pistillate primary flower which is generally fertile. A more extensive study of such types as these may throw light on the origin of dioeciousness in the genus Fragaria.

Staminate × Staminate

The cross staminate × staminate should give results identical, so far as somatic flower types are concerned, with the combinations thus far considered. Only a single combination of this type was studied. The female parent was a staminate plant of *F. virginiana* which produced a single pistillate primary flower. This, when pollinated with pollen from a staminate *F. virginiana*, set a few seeds. These produced 4 plants, all of which were somatic hermaphrodites or males. The cross was truly staminate × staminate rather than hermaphrodite × staminate, as the plant used as the female would always be considered a pure staminate in nature. Thus far we have obtained a total of 1,714 hermaphrodites or somatic hermaphrodites to a possible 38 pistillates when hermaphrodites were pollinated from hermaphrodites in 25 different combinations. In view of the explanation given of the probable sex condition of the 38 pistillates, the results leave little doubt that hermaphrodites or somatic hermaphrodites carry only the hermaphrodite and male determiners and do not contain the determiner for femaleness. The strawberry is then in direct contrast to the condition found in Lychnis and Vitis, in both of which the hermaphrodites have been shown to be heterozygous for the sex determiners.

Pistillate × Hermaphrodite

Table 3. *Results of Crossing Pistillate with Perfect-flowered Varieties of Strawberry*

Lot No.	Varieties Crossed	Sex of F_1 Seedlings		
		Hermaphrodite	Pistillate	Staminate
28/16.	(F_1 of Crescent × ?) × Minn. no. 3	21	16	
40/16.	Enormous × Dunlap* Bederwood × Glenville* (7 plants)	14	11	
40/16.	Enormous × Dunlap	5	7	
40/16.	Enormous × Dunlap* 5/15 67–2 ♀ × Glenville*	13	14	
66/16.	Columbia × 5/15 70–4	26	27	
15/15.	*F. virginiana* × 778	18	18	
33/16.	5/15 62–5 × Glenville	27	37	
45/16.	5/15 64–2 × Glenville	32	28	
49/16.	Bederwood × Glenville	26	28	
65/16.	Columbus × Glenville	9	10	
56/16.	5/15 × Glenville	8	5	
	Total	199	201	

* Plants mixed by acident.

If we accept the chromosome theory of inheritance as applied to sex in the strawberry, we must conclude that the females are heterozygous for the determiners *H* or *M* and *F*, depending on whether cultivated varieties or wild pistillates are concerned, in view of the fact that the male plants and the hermaphrodites have both been shown to be homozygous for sex determiners.

The results of crosses in which pistillates were used as the female parent and various hermaphrodites as the male parent are given in table 3. There is a definite 1 : 1 segregation of the 400 seedlings into 199 hermaphrodites and 201 pistillates.

In four crosses of pistillate × hermaphrodite made by Mr. Charles Haralson and recorded by the writer, the results shown in table 4 were obtained.

Table 4. *Results of Crossing Pistillate with Perfect-flowered Varieties of Strawberry*

	Pistillate	Hermaphrodite
Seedling 1020 ♀ × Progressive	31	41
Seedling 1020 ♀ × Seedling 907	16	9
Meteor ♀ × Seedling 907	28	22
Productive ♀ × Seedling 1017	4	5
Total	79	77

These results leave little doubt that the pistillate plants of Fragaria are heterozygous with respect to the sex-determining factors. The results of the writer in crossing pistillates by hermaphrodites are in keeping with the

results obtained by Richardson (2), who obtained a total of 203 ♀ and 173 ♂ or ☿ in making similar crosses.

Sex inheritance, in all these crosses, conforms to the theory of a heterozygous condition of the females, the female condition being completely dominant, with one possible exception. This was the cross 45/16 in which 5/15 64–2, a pistillate, derived from the cross 5/15 (table 3) was used as the female parent and Glenville as the male parent. This cross resulted in 32 hermaphrodites and 28 females and approximated the expected 1 : 1 ratio. The fertility of the seedlings, however, was not what was expected. Fifteen of the females set all flowers except an occasional late one but produced only a few achenes per berry, and as a consequence all berries were nubbins. The remaining 13 females were completely fertile except for an occasional late flower. Twelve of the hermaphrodites set some fruit and in some cases nearly as many flowers as the females, but all the berries were likewise nubbins. Eleven hermaphrodites set nearly all flowers and the berries were perfect; 9 others set one half or less of the flowers perfectly. The remainder were males. The expectation from this cross was 1 fertile female to 1 hermaphrodite varying in degree of fertility. In other words, the females should all have been completely fertile and the hermaphrodites either fertile or partially sterile depending on whether the *H* from the female parent united with the more fertile determiner of Glenville or with the one which has been shown to be practically sterile; and on the extent to which the *H* determiner from the female parent is dominant over the sterile *H*. Instead of the expected ratio, 1 normal female to 1 practically sterile female (nubbins) to 1 fertile hermaphrodite to 1 partially fertile hermaphrodite was obtained. This and one other are the only cases observed in which females have not been nearly completely fertile. There has evidently been a decided change in the female determiner in this single instance. For an explanation of this peculiar condition it seems we must go back beyond the two immediate parents. The male parent Glenville is probably not the cause, as, when crossed with other females, it has not produced similar results. The female parent was the offspring of a wild female × hermaphrodite 778. Two other F_1 females of this cross acted according to expectation when crossed with Glenville (56/16 and 33/16, table 3). It seems, therefore, that we are forced to assume that a change took place, probably during the reduction division, in the wild female grandparent. A crossing over in the female and male chromosomes between the female determiner and the suppressed male determiner and *vice versa* in the male chromosome would give a chromosome in which both sexes are suppressed: (*mf* or *h*). An egg containing such a chromosome if fertilized by a male gamete from 778 carrying an hermaphrodite determiner would produce an individual having one determiner for femaleness and one for maleness, these two being linked (*H*). The constitution of this individual would then be *Hh*. We have already seen that a single dose of femaleness is

sufficient to produce fertile pistils (wild female) while one dose of maleness is not sufficient to produce functional stamens. This plant would then be a female. The only records on this individual, which was grown in the greenhouse, stated that it was female and had berries strikingly like the berries borne on the original wild female. The berries borne on its sibs were all much of a type and more intermediate between its parents, although most F_1 plants of *F. virginiana*, when a pistillate is used as a female parent, resemble the wild to quite a degree. If we are correct in the assumed constitution of this female, and if it is crossed with Glenville, the expected combinations would be as follows: *hH* (female) × *HH*^{1} (Glenville in which *H* is a normal hermaphrodite and *H*^{1} is a weak hermaphrodite or male) = 1 *hH* (a fertile female) : 1 *hH*^{1} (a female sterile or producing only nubbins): 1 *HH* (a fertile hermaphrodite) : 1 *HH*^{1} (a partially fertile or sterile hermaphrodite of the Glenville type). These are in fact the types which were produced. A similar explanation might be given for the origin of the completely sterile wild clones of *F. virginiana* which are occasionally found in which neither the stamens nor the pistils are functional. It is not maintained that this is the correct explanation of the results obtained. It is based on the assumption that the male and female determiners in an hermaphroditic chromosome are separate and distinct, and that, as a consequence, crossovers might occur between them. The four types of plants were very distinct, and the numbers were so large as to indicate a genetic condition different from any previously studied.[2]

Although the results thus far given all point to the hermaphrodites being homozygous for sex determiners, and the pistillates heterozygous, they are not absolutely conclusive, since the progeny of selfed females have not yet been studied. This I believe will be impossible in the strawberry, as I have never found a stamen on any of the cultivated pistillate varieties or on wild pistillate clones which showed any signs of producing pollen. If, however, we can transfer the sterile male condition of the wild *F. virginiana* to progeny through the use of a wild *F. virginiana* female with fertile hermaphrodites, it would seem that we have proved its heterozygous condition for *F* and *M*. This seems to have been done in the cross 5/15 (table 3) in which a wild *F. virginiana* ♀ was the pistillate parent and the very fertile hermaphrodite 778 was the male parent. This cross produced 18 pistillates, all fertile to a high degree, and 18 hermaphrodites. Nine of the hermaphrodites were completely fertile, 4 were fertile except for an occasional tertiary or quaternary flower, while 5 set only an occasional fruit. On the theory that pistillates are heterozygous, the genetic constitution of the wild female would be *FM* and that of the hermaphrodite *HH*^{1}. The female progeny of this cross would then all be *FH* or *FH*^{1} and would be completely fertile. The constitution of the hermaphroditic progeny would

[2] The other case in which a female was nearly sterile resulted from the cross 65/16, Columbus × Glenville, which produced 9 hermaphrodites and 11 females, 10 of which were completely fertile while one produced only nubbins.

be HM and H^1M, depending on which of the H chromosomes of 778 combined with the M. What should be the sex of these new genotypes? They should be hermaphrodites as far as somatic condition is concerned and should be completely fertile, partially sterile, or sterile, depending on the extent to which their sex is determined by the hermaphrodite or male determiners and on whether the two hermaphrodite determiners have an equal or an unequal potential fertility. The hermaphroditic progeny were of two classes consisting of 9 completely fertile clones and 9 partially sterile, as was previously mentioned. These results would seem to indicate that the two H factors of 778 differed slightly in potential fertility and, when in combination with the same male factor, resulted in different degrees of fertility. This does not necessarily follow, however, as it will be shown later that a single H factor of a female plant, when united with the two male factors of a wild male, produced progeny showing the same variations in fertility. The result, when one of the 5/15 (*F. virginiana* ♀ × 778) hermaphrodites was selfed, was reported in table 1, 57/16, and was analyzed in the following discussion. It was there shown that pure males, completely fertile hermaphrodites, and intermediates could be produced from one of these HM hermaphrodites. These results prove that the wild females carry the factor for maleness, femaleness being completely dominant over it, and that the factor for maleness can be transferred through the wild female to hermaphroditic progeny, and that, when in combination with an hermaphrodite factor, either one may act as a dominant, producing complete fertility or sterility, or both may show partial dominance resulting in partial fertility.[3]

The question as to whether one hermaphrodite of the constitution HM may be fertile and another partially sterile, or whether one H factor may be potentially more dominant than another, is extremely important from an economic as well as from a scientific standpoint. The case of Glenville, previously mentioned, in which the sex varied from male to hermaphrodite under different cultural conditions, and which produced, when selfed, both fertile and sterile seedlings, is evidence that one of the sex factors may be dominant at one time and the other dominant under other conditions. Further studies on the relative dominance and potential fertility of the H factors in the cultivated strawberry may throw considerable light on the reasons why certain varieties fruit heavily under some conditions and produce an inferior grade of irregularly shaped fruit under other conditions.

Pistillate × Staminate

The cross pistillate × staminate should give the same results as pistillate × hermaphrodite as far as somatic appearances are concerned. The

[3] Both the H and the M factors must be considered to be recessives, as both are recessive to the F factor. This seems to be true in spite of the fact that the H factor appears to carry functional F and M factors linked.

progeny of only a single cross of this type has been studied. The female parent used was a pistillate derived from crossing a wild pistillate *F. virginiana* (*FM*) by the hermaphrodite 778 (*HH*) (5/15, table 3). All pistillates from such a cross should have the constitution *FH*. This female was crossed with a wild *F. virginiana* staminate. Twenty F_1 seedlings were obtained. Eleven of these were pistillate and 9 hermaphrodites or somatic hermaphrodites, thus giving a close approximation to the expected 1 : 1 ratio. The pistillates were all very fertile. Of the 9 hermaphrodites obtained, 3 set no fruit while 6 set fruit in varying amounts, from only a nubbin on the primary flower with the others sterile, to some which set fruit on flowers of all degrees but with a portion of the tertiaries and quaternaries sterile. If the 9 hermaphrodites are divided into two groups with regard to whether sterile flowers or fertile flowers predominate, there will be 6 classed as sterile and 3 as fertile. These plants are all of the constitution *MH*, the *H* factors all being identical and the *M* factors being derived from the wild male parent. Thus the male factor of the wild female was replaced by an hermaphrodite factor in the female parent of this cross, and maleness was introduced into the progeny by a wild male (*MM*). The results of this cross are similar with respect to fertility of the hermaphrodites to those obtained from cross 5/15 in which a wild female (*FM*) was crossed with a cultivated hermaphrodite (*HH*). It is thus seen that maleness may be introduced into otherwise fertile strains of strawberry by the use of either the wild females or the wild males. Even though the flower types with respect to fertility of the hermaphrodites are not always clear-cut, these results are in accord with the chromosome theory of sex inheritance. It would seem that the outward expression of a given sex determiner may be influenced by the sex determiner with which it is associated and also by the autosomes associated with the sex chromosomes in the nucleus.

Discussion

The Sex Determiners

In the above presentation of data, and in the conclusions which have been drawn from them, it has been assumed that the determiners for sex are definite factors and that they are carried in a definite pair of sex chromosomes. We must assume that the various sex conditions which appear have been derived originally from an hermaphroditic condition in which the determiners for the two sexes are linked in each of a pair of chromosomes. The various sex types which appear in either the grape or the strawberry, or, in fact, in any of the flowering plants which are dioecious and show a variety of sex types intermediate between staminate and pistillate, we assume to have been derived by suppression, either partial or complete, of one of the determiners for sex in the sex chromosomes, leaving the other factor functional (5). It can hardly be assumed that one or the other sex

determiner is ever completely eliminated, since abortive organs, either stamens or pistils, are often present in flowers of the opposite sex in supposedly strictly dioecious forms, and occasionally even perfect reproductive organs of the opposite sex are found in such "strictly" dioecious forms as *Acer Negundo*.

In the strawberry the assumption of partial suppression of the female determiner is necessary in both the sex chromosomes of male plants of *F. virginiana*. Femaleness is still present and functional to the extent of producing apparently normal pistils, which, however, prove to be sterile. In the hermaphrodites of the cultivated varieties (derived from dioecious wild species) suppression of femaleness is very slight but is still present to a degree in many cases, as shown by the decreased fertility of such forms when compared with pistillate varieties (7). In some hermaphrodites there is apparently no suppression of femaleness, since they may be completely fertile. The pistillate plants of *F. virginiana* we could assume to contain two sex chromosomes which are different. One of these would be identical with the two found in wild staminates, *i.e.*, male (+ female suppressed), and the other bearing the normal female determiner linked with a suppressed male determiner. Cultivated pistillate varieties would then be of the constitution female (+ suppressed male), and hermaphrodite. In other words, we should assume that a single dose of femaleness, if carried as an *F* factor, is sufficient to produce fertile pistils in the pistillate plants, while two doses of maleness are necessary for the production of functional stamens. If only a single dose is present, staminodes are produced. On the other hand, if there is present a single dose of femaleness linked with a normal male determiner, thus forming the recessive *H* factor, it may or may not be sufficient to produce functional pistils.

An interpretation of the data presented on the basis of these assumed chromosome conditions leaves little doubt that the males and hermaphrodites are homozygous for sex chromosomes bearing functional male determiners. The females, on the other hand, must be assumed to possess one sex chromosome carrying a male or hermaphrodite determiner, and another chromosome carrying the female determiner. In other words, we have a condition similar to that existing in pigeons and cultivated fowl in which the males are apparently homozygous and the females heterozygous for the sex determiners. The condition is opposite to that which has been found to exist in the females of Bryonia, Lychnis, sweet pea, and Vitis. In view of the fact that either males or females may be heterozygous in the animal kingdom, it is not surprising that both types should be found in plants.

The writer has not attempted to give a review of the literature on sex inheritance and sex determination in plants, since this has recently been done rather comprehensively by Yampolsky (8, 9, 10).

Although a factorial basis for sex determination is rather generally admitted to be correct for explaining inheritance of sex in animals, Yampol-

sky and others are not inclined to believe that it will also explain the conditions found in plants. This is due primarily to the fact that an individual may during its life or during the flowering of a single cluster appear to change its sex. In other words, there may be "a periodic alternation of sex." Yampolsky (10, p. 99) holds that "a factorial hypothesis for sex can not explain these results." In his work on sex intergrades and sex inheritance he worked primarily with *Mercurialis annua*. The material is unfitted for a study which will readily produce accurate results because of the mechanical difficulties encountered in making the crosses necessary for a proper interpretation of the genetic constitution of the several plant types. His conclusion that males when selfed tend to produce males or plants predominantly male, and that females selfed tend to produce females or predominantly females was based on the results of selfing a large number of flowers on female plants, of crossing females with males (which produced a 1 : 1 ratio of males and females), and of selfing female flowers on male plants. His results obtained by selfing female plants would seem to show conclusively that the females are homozygous for sex determiners, if we attempt to explain the results on the factorial basis. Females × males produced a 1 : 1 ratio of females and males, as would be expected, and the results suggest, in view of the results obtained from selfed females, that the males are heterozygous for the sex determiners. Although self-pollinated males produced only males, the statement (8, p. 434) that "the selfed males of *Mercurialis annua* may be said to record their own gametic constitution" is not based on sufficient evidence to warrant discarding the chromosome hypothesis of sex inheritance in plants. Yampolsky does not take into consideration the possible effect of lethal factors on sex ratios. There is abundant evidence from his results to indicate that the sex ratios of the progeny of his selfed males may have been influenced by lethal factors which inhibited the normal development of the embryos or which later affected germination either by weakening it or by destroying it completely. From a total of 156 female flowers on one lot of male plants he obtained 283 seeds. Of these, 31 were immature. He explained immaturity as the result of one seed developing faster than the other in two-seeded ovaries, so that in gathering the seeds the immature one is likely to be gathered with the mature one. Of the 283 seeds obtained from this lot of male plants, 219 were sown. Of this number *75 seedlings only developed*. These were all male plants. It is obvious that something interfered with the normal development of the other seeds. Certain ones developed slowly; others germinated weakly and then died; while others did not germinate at all. Horticulturists are generally aware of the fact that slightly immature seeds germinate nearly as well as, and often better than, mature ones. There is a question as to whether immaturity can be correctly assigned as the cause of failure of any of the seeds to germinate. Yampolsky (8, p. 432) has used the work of Shull (4) on irregular sex ratios in *Lychnis*

dioica as an argument against a Mendelian explanation of sex inheritance, stating that "the Mendelian hypothesis of sex in itself can not account for the preponderance of one sex to the exclusion of the other." The writer (7, p. 663), however, has shown that wherever in Shull's work (3, 4) irregular sex ratios were obtained, they were the result of partial or complete elimination of the male (and to a slight extent of the female) gametes which carried the mutant factor "narrow leaf" linked with femaleness (or with hermaphroditeness in the case of *Melandrium album*). This explanation was borne out by the presence of large amounts of abortive pollen in the narrow-leaved Lychnis male. Dorsey (1), working with plums, has expanded the idea of gametic elimination due to unfavorable genetic combinations to include the elimination of embryos during various stages of their development because of unfavorable genetic combinations and has given considerable evidence in support of this explanation as the cause of dropping of plums through the summer and of poor germination of apparently normal seeds from certain crosses.

It appears from Yampolsky's results with seed of selfed males that immaturity and lack of germination in the seeds from selfed males may be due to unfavorable genetic combinations which allowed development of certain embryos to proceed only to a certain point, when growth ceased. In his table 6 (8, p. 422) are reported a total of 156 flowers which produced 283 seeds on male plants. Besides these there were produced on the same plants "approximately 90 other female flowers . . . which failed to develop seeds and dropped off." A total of 492 seeds might then have been expected if all had set and developed normally; 283 were actually produced, and of these, 31 were immature, 5 germinated weakly, 139 did not germinate, and 26 were not planted. Certainly there is evidence of elimination in these results, and they can not therefore be taken as indicating that the genetic condition of the males of Mercurialis is pure male and not heterozygous for male and female determiners, as would be indicated by the results of crossing females with males. All possible genetic combinations should be thoroughly tested before conclusions as to the genetic conditions with respect to sex may be safely arrived at. The species in question makes such a study extremely difficult.

In Fragaria, as in many other forms, the sex of all the flowers of any given plant is not necessarily the same. It is quite common in the perfect-flowered varieties for practically all the earliest flowers to be female. In some clones this condition has been exaggerated to a point at which only a few flowers produce stamens while the remainder are female. Male flowers on hermaphroditic varieties are common, and are practically always limited to the last flowers of a cluster to bloom. Female flowers may be found in the primary position of the inflorescence of wild males, and in such cases generally set fruit. Completely sterile flowers are not uncommon among the later flowers of a female inflorescence.

In general, in Fragaria there appears to be a tendency towards femaleness in the earliest flowers which bloom; while the later flowers have a tendency towards maleness in the hermaphroditic varieties. A similar tendency with regard to increasing pistil sterility in the later blossoms is apparent, but to a lesser degree, in the cultivated females. The females of dioecious wild forms and all the plants of the wild hermaphroditic forms exhibit an extremely high degree of pistil fertility extending through all the flowers of a cluster. It is seldom that irregular berries are seen in wild forms, even under the most adverse conditions.

The writer does not believe that the presence of other sex types of flowers on a plant, which is predominantly of one sex, necessarily means that sex is not determined by specific factors or that such a condition is an argument against a Mendelian interpretation of sex in plants.

If it is assumed that dioecious forms have been derived from hermaphroditic forms, and if we accept, for the present, the theory that in hermaphrodites the two sex determiners are linked in one chromosome, then the production of males and females in such forms as Fragaria and Vitis, where the opposite organs are still present but not functional, must have come about by the partial suppression of one or the other sex condition in the chromosomes but not by its complete loss. Thus, in the female plant of *F. virginiana* two sex chromosomes would be present: one which carries the functional factor for femaleness (which is sufficient to produce functional pistils), and another which carries the functional factor for stamen production, but which, in the absence of another similar functional factor, is unable to produce functional stamens. Each chromosome, however, would carry the opposite factor suppressed. The males would then contain two like chromosomes, each of which carries a functional determiner for stamen production and a suppressed determiner for femaleness. It appears that no other assumption will meet the actual conditions. It is apparent from the facts previously given that the sex condition may be varied by varying the conditions under which the plants are produced. This may result by changing either the outer environmental factors of the plant or the internal factors of nutrition (?) due to the position of the flowers on the inflorescence. Such changes have been observed. It is not a far step, then, to assume that in certain parts of a plant conditions are set up which have a tendency to decrease the suppression of factors which are already present and to allow flowers or flowering parts of the opposite sex to be produced on a given plant. The determiners for sex in plants may be specific entities, but still their full or partial expression may depend upon the immediate conditions surrounding them at the time of flower production.

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SOME EFFECTS OF PHYSIOLOGICAL CONDITIONS ON GENETIC CHARACTERS OF WHEAT

W. F. GERICKE

(Received for publication September 20, 1922)

The relative earliness or lateness of maturation of wheat (comparing one variety with another) and the awned or awnless form of the spikelets are two classes of genetic characters used by agronomists to distinguish different varieties. Under the usual conditions of field culture, such as prevail in any wheat-growing section, these characters have a considerable degree of fixity in pure-line strains. That they are not immutable, but change with conditions that concern both the nutrition and the external environment of the plants, has become apparent from some recent investigations of the writer.

Pertaining to that investigation, nine different varieties of spring wheat, representing a range from early to late wheats, were planted and grown to maturity in a fertile soil. The seeding was made December 15, and the cultures were exposed to greenhouse conditions for the entire growing period of the plants. They ripened in due course of time, and in such order and with such characters as would be expected from the known behavior and properties of these wheats. The earliest variety matured in the last week of April and the latest seven weeks thereafter.

On March 20, another test was begun with these same varieties, but using tap water as the growth medium. Previous tests by the writer had shown that wheat could be grown to maturity in tap water. This medium is markedly deficient in the essential salt nutrients, the total salt concentration being approximately 0.12 atmospheres osmotic value. The usual method employed for culture solution experimentation was followed in these tests. This consisted in germinating the seed on cloth netting suspended over, and in contact with, water. When the plants were 6 to 8 cm. high, they were set into cork supports fitted in two-quart containers (Mason jars) filled with tap water. Twenty cultures of five plants each were used for each variety tested. All cultures were then subjected to the same climatic conditions, being placed on a centrally located table in the greenhouse and there allowed to grow as they would without further treatment. There was no renewal of tap water during the entire growing period,

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and only once, when the cultures were six weeks old, was there an addition of distilled water ranging from 300 to 500 cc. per culture to make up for the loss in transpiration of water by the plants. One half cc. of 0.01 molecular solution of $FeSO_4$ was added to each culture at the beginning of the experiment, as it was known that, if a small trace of iron was not added to the tap water, the culture would soon turn yellow and die. All the varieties matured and produced grain. The first variety to mature ripened during the last week of June, and the last during the first week of August. The amount of grain produced obviously was small. The yield differed markedly with the varieties, but the results were consistent for each variety. These differences in yield of grain obtained appear to bear in no small measure upon a principle that determines adaptation of varieties of wheat (and perhaps of other species of plants) to the supply of nutrients in the growth medium. This phase of the experiment will be treated in another paper.

It was found, when the results of the two tests were compared, that the order of the maturity of the varieties was not the same in the two cases. The variety that ripened first of those grown in soil was the fifth of the varieties to mature when grown in tap water. Therefore, the effect of tap water, a poor growth medium, deficient in the essential salt nutrients, plus whatever effect the then prevailing climatic complex had, was to permit the alteration of the order of maturity of the varieties from that obtained by growing wheat in fertile soil. The obvious result of this change was to make a wheat, which was early under a given set of conditions, relatively late under the other set of conditions. Or, stated in another way, physio-

TABLE 1. *Differences in Order of Maturity and Change in Awned Characters of Different Varieties of Wheat, Grown in Soils and Tap Water (Medium Deficient in Nutrients)*

Name of Variety	Order of Maturity		Change of Awned Characters
	Soil	Tap Water	
Bunyip	1	5	No change
Cedar	2	1	No change
Hard Federation	3	4	No change
Fulcaster	4	3	No change
Sonora	5	2	7 percent of the plants produced awns when grown in tap water; all awnless in soil cultures
Early Baart	6	6	No change
Dart's Imperial	7	7	No change
White Australian	8	9	No change
Marquis	9	8	No change

logical conditions had a profound influence in determining whether certain varieties of wheat become relatively early or relatively late.

Another genetic character of wheat altered by growing the plants in

tap water from that which prevailed on plants grown in soils was the appearance of awns on a so-called awnless variety (Sonora). Seven plants out of the one hundred produced heads having awns one and one half to two inches long, whereas none of the plants grown in soils produced awned spikelets, other than the rudimentary organs two eighths to three eighths inch long common to that variety. Tap water, and perhaps also the effect of the then prevailing climatic complex, therefore served as a means to permit expression of a character in wheat that did not appear under the usual conditions in which this variety is grown.

As a much fuller account of the subject matter will appear in due time elsewhere, only a short table of data (table 1) is given herewith.

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A QUANTITATIVE STUDY OF ANISOPHYLLY IN ACER

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The fact that lateral or horizontal shoots often differ from vertical ones in showing a dorsiventral as opposed to a radial structure has long been observed. One aspect of this dorsiventrality in many species is the difference in size, and often in shape, between leaves on the upper and those on the lower sides of the branch. To this phenomenon the term "anisophylly" has been applied by Wiesner. The upper leaves are generally smaller than the lower ones, and those arising from the sides of the shoot are intermediate in size. There has been much discussion as to the factors which produce anisophylly—whether gravity, light, or various internal conditions are responsible—and the whole problem is of particular interest to students of morphogenesis in that it provides a fairly simple case for an analysis of some of the factors which determine form.

Wiesner (1868) and Frank (1868) were the first to consider the problem, and both believed anisophylly to be due primarily to gravity. In Picea and Acer, Frank twisted horizontal twigs, still attached to the plant, through an angle of 180° and tied them there. In Picea, the original anisophylly was maintained in the new growth, the leaves now on the upper side still being the longer, but the difference was not as marked as before. In Acer there was a complete reversal, the originally upper leaves, now on the lower side, being much longer than the originally lower ones. He found that anisophylly was not as marked in shoots grown in a dark chamber, thus suggesting that light might also play a part. Kny (1873) repeated Frank's experiment, using Abies instead, and found that the original anisophylly was finally reversed in the second season following the turning of the twigs. Frank (1873) also found that in shoots of Thuya there is a very definite anatomical difference between the upper and the lower sides. This difference was reversed when the twig was twisted through 180°; but as a result of shading experiments and on the basis of other evidence, Frank concluded that light, rather than gravity or an internal tendency toward bilaterality, was the factor involved. Goebel (1880) first suggested that internal factors were also at work in producing anisophylly. Rosenvinge (1889) showed that dorsiventrality may sometimes be due to external and sometimes to internal factors.

Wiesner in his later papers emphasizes more strongly the importance of light. At first he regarded the large size and long petioles of leaves on the lower sides of anisophyllous shoots as due to etiolation. Later (1894),

he concluded that these leaves actually got more light and thus grew larger than the upper ones. He also noticed that it was always the leaf on the abaxial side of the twig which grew larger, and for this general tendency for structures on the outside of lateral shoots to become larger than those on the inside, next the mother axis, he proposed the term "exotrophy" (1892). He believed this to be due to the better nutrition of these outwardly placed structures, and suggested that exotrophy might be an important factor in anisophylly. Weisse (1895), working with Acer, showed by shading experiments that the leaves which were shaded were smaller than those in the light. He also put young maples on a horizontal clinostat, thus eliminating the effect of gravity, and found that, although anisophylly persisted somewhat, it was less marked than in the control plants. Weisse stresses gravity rather than light as a factor in anisophylly. He agrees with Wiesner that exotrophy is also concerned, but does not believe that nutrition is the cause of it. He renamed the phenomenon "ectauxesis," and believed it to be due to inherited morphological factors and perhaps to be teleological. Figdor (1897) caused the side shoots of eight species to grow vertically; later, he (1904) planted a young maple tree in such a position that one of the side branches was vertical, and in this position these lateral shoots ultimately lost their anisophylly. Vertical shoots bent downward often acquired it. Figdor believes that gravity, light, and possibly certain internal factors are at work. Nordhausen (1902) regards light as an important factor in leaf asymmetry and anisophylly in Aesculus, where those portions of the leaf-blade presumably exposed to greater light are thicker than the rest; but holds that light plays but a small part in the case of Acer. He regards exotrophy as an important factor. Heinricher (1910) concludes that differences in transpiration rate, governed by temperature, are important in producing anisophylly in Sempervivum. This is denied by Doposcheg-Uhlár (1913), who attributes conditions in this genus to light and gravity only. Boshart (1911) believes that leaf asymmetry (and presumably other phenomena of anisophylly) are controlled chiefly by conditions at the growing point, such as the space occupied by the leaf primordia and their relation to the rest of the meristem. A survey of the literature up to 1909 is presented by Figdor (1909).

A number of factors may evidently be concerned with the production of anisophylly, but there is no unanimity of opinion as to the part played by each. The purpose of this paper is to present results of a statistical study of leaf measurements on anisophyllous twigs of Acer, the genus which has been most frequently studied in this connection, for the purpose of reducing the problem to a quantitative basis and in the hope that a knowledge of the relative dimensions, shape, and variability of leaves occupying different positions on the lateral shoots may produce evidence of value.

Maple twigs growing horizontally show marked anisophylly. The upper member of each vertically oriented pair is distinctly smaller than the lower

and differs from it somewhat in shape. The members of the horizontally oriented pair are intermediate in most characters between the upper and the lower members of the vertical pair. They are also asymmetrical, the lower one of the two main lateral veins being longer than the upper.

1000 leaves of *Acer saccharum*, growing on twigs which were horizontal or essentially so, were studied. In order to avoid possible genetic differences, they were all taken from a single tree of this species. Only those twigs were chosen in which the successive leaf pairs were clearly vertical and horizontal, all those in which the pairs were at all oblique in their insertion being eliminated. Twigs were chosen from all parts of the crown of the tree. All the leaves on each twig were recorded, so that there is an equal representation of those from the basal, median, and terminal regions of the year's growth. Leaves were harvested in midsummer after complete maturity had been reached. Length of petiole, length of midrib, width of blade from tip to tip of the lateral lobes, and length of right and left main

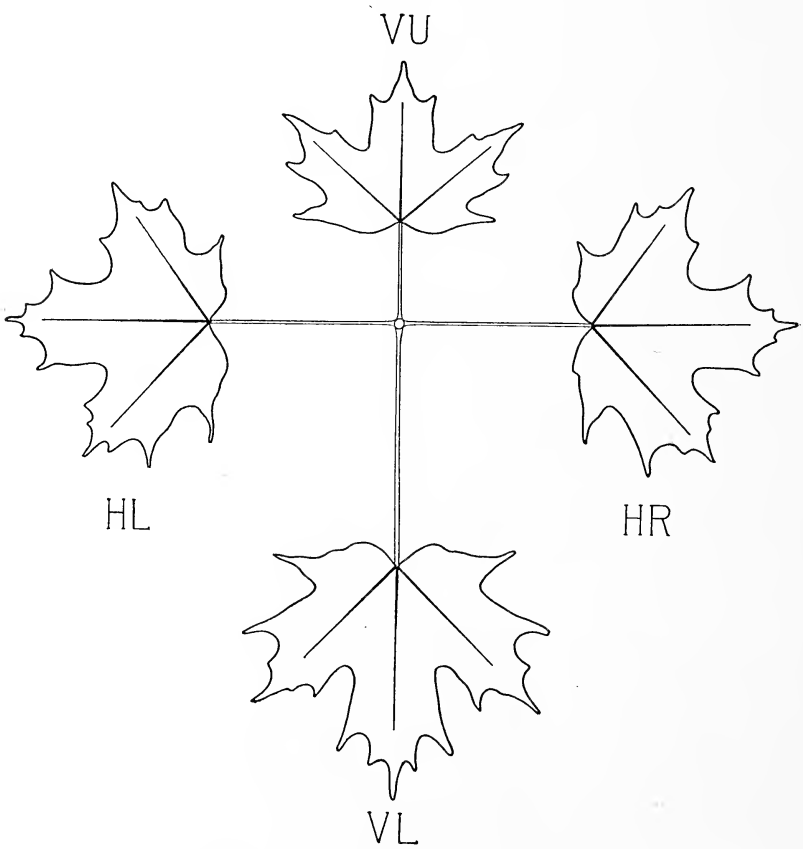

FIG. 1. Diagram representing the four groups of leaves, dimensions proportional to the means in table 1. VU, vertical upper leaf; VL, vertical lower; HR, horizontal right; HL, horizontal left.

TABLE 1. *Mean, Standard Deviation, and Coefficient of Variability for the Various Size Characters in the Total Population and in the Four Leaf Groups*

	M.	σ	C.V.	Number
Length of Midrib (cm.)				
Total	*9.50 ± .059*	*2.75 ± .041*	*29.0 ± .508*	*1000*
Vertical upper	7.72 ± .102	2.44 ± .075	31.6 ± 1.106	258
Vertical lower	10.59 ± .114	2.73 ± .081	25.7 ± .858	258
Horizontal right	9.86 ± .103	2.37 ± .072	24.0 ± .815	242
Horizontal left	9.89 ± .108	2.48 ± .076	25.0 ± .858	242
Length of Right Rib (cm.)				
Total	*8.19 ± .055*	*2.79 ± .042*	*34.1 ± .620*	*1000*
Vertical upper	6.85 ± .102	2.43 ± .072	35.6 ± 1.301	258
Vertical lower	9.07 ± .123	2.94 ± .087	32.4 ± 1.145	258
Horizontal right	8.85 ± .117	2.70 ± .083	30.5 ± 1.094	242
Horizontal left	7.98 ± .109	2.51 ± .077	31.5 ± 1.140	242
Length of Left Rib (cm.)				
Total	*8.23 ± .060*	*2.81 ± .042*	*34.2 ± .621*	*1000*
Vertical upper	6.86 ± .101	2.41 ± .071	35.1 ± 1.271	258
Vertical lower	9.12 ± .124	2.97 ± .088	32.5 ± 1.150	258
Horizontal right	8.06 ± .108	2.49 ± .076	30.9 ± 1.116	242
Horizontal left	8.80 ± .118	2.72 ± .084	30.9 ± 1.116	242
Width of Blade (cm.)				
Total	*12.86 ± .084*	*3.94 ± .059*	*30.6 ± .539*	*1000*
Vertical upper	11.18 ± .153	3.65 ± .108	32.7 ± 1.165	258
Vertical lower	13.72 ± .177	4.22 ± .126	30.7 ± 1.067	258
Horizontal right	13.31 ± .159	3.65 ± .112	27.4 ± .957	242
Horizontal left	13.19 ± .161	3.71 ± .114	28.1 ± .989	242
Thickness of Blade (mm.)				
Total	*.1607 ± .0004*	*.0201 ± .0003*	*12.5 ± .196*	*1000*
Vertical upper	.1609 ± .0008	.0200 ± .0006	12.4 ± .380	258
Vertical lower	.1608 ± .0008	.0195 ± .0006	12.1 ± .369	258
Horizontal right	.1603 ± .0008	.0190 ± .0006	11.8 ± .373	242
Horizontal left	.1614 ± .0009	.0202 ± .0006	12.5 ± .394	242
Area of Blade (sq. cm.)				
Total	*64.84 ± .734*	*36.13 ± .528*	*55.7 ± 1.257*	*1008*
Vertical upper	47.60 ± 1.090	25.99 ± .770	54.6 ± 2.429	258
Vertical lower	78.90 ± 1.713	40.81 ± 1.213	51.7 ± 2.210	258
Horizontal right	70.20 ± 1.485	34.30 ± 1.050	48.9 ± 2.099	242
Horizontal left	68.40 ± 1.448	33.20 ± 1.016	48.5 ± 2.068	242
Volume of Blade (cc.)				
Total	*1.049 ± .0127*	*.594 ± .0089*	*56.6 ± 1.281*	*1000*
Vertical upper	.757 ± .0189	.439 ± .0135	58.0 ± 2.648	258
Vertical lower	1.242 ± .0280	.668 ± .0198	53.8 ± 2.352	258
Horizontal right	1.112 ± .0239	.553 ± .0168	49.7 ± 2.145	242
Horizontal left	1.071 ± .0241	.555 ± .0170	51.8 ± 2.291	242
Length of Petiole (cm.)				
Total	*8.35 ± .099*	*4.64 ± .070*	*55.5 ± 1.245*	*1000*
Vertical upper	4.58 ± .093	2.21 ± .066	48.2 ± 1.988	258
Vertical lower	11.17 ± .213	5.07 ± .151	45.4 ± 1.823	258
Horizontal right	8.85 ± .174	4.00 ± .123	45.2 ± 1.816	242
Horizontal left	8.89 ± .177	4.08 ± .125	45.9 ± 1.918	242
Diameter of Petiole (mm.)				
Total	*1.509 ± .0091*	*.425 ± .0064*	*28.2 ± .489*	*1000*
Vertical upper	1.236 ± .0142	.337 ± .0100	27.2 ± .920	258
Vertical lower	1.679 ± .0187	.437 ± .0130	26.0 ± .873	258
Horizontal right	1.569 ± .0174	.401 ± .0123	25.6 ± .883	242
Horizontal left	1.572 ± .0164	.378 ± .0116	24.0 ± .819	242

lateral veins (as seen from the upper or ventral leaf surface) were recorded. Petiole diameter was measured at the mid-region of the petiole by a micrometer caliper. Blade thickness was also measured by a micrometer caliper at two symmetrically situated points at right and left of the midrib in a region where there were no projecting veins and where mesophyll thickness alone could be determined. These two measurements were almost always identical, but when different were averaged. Blade area was determined by outlining the blade on standard-weight paper, cutting this out, and weighing the cut-outs. Blade volume was calculated by multiplying area by thickness.

Constants were determined for the whole population and for each of the four groups into which it was divided: vertical upper and vertical lower—the upper and lower members of the vertically oriented leaf pairs; and horizontal right and horizontal left—the right and left members of the horizontally oriented pairs as seen by an observer facing the apex of the twig (fig. 1). These constants are set forth in table 1.

An inspection of this table brings out certain facts as to the differences between these leaf groups in *dimensions*, *shape*, and *variability*.

Differences in Dimensions

A comparative study of the means shows that in all measurements (except blade thickness) the vertical upper leaves are markedly smaller than the lower ones, and that the members of the horizontal pairs are in all cases intermediate between these two. A significant fact which the figures bring out is that the combined size of the two members of the vertical pairs approximately equals the combined size of the two members of the horizontal pairs. In other words, the plant has a given amount of leaf material available at a given node (of course this amount decreases as we approach the apex of the twig), but this material is divided between the two leaves at this node in varying proportions, depending on the orientation of the particular leaf pair which arises there.

Perhaps the most striking fact brought out is that the four leaf groups are of almost exactly the same blade thickness. Whatever are the factors which produce the differences between these groups in other dimensions, they evidently do not affect this one. This uniformity cannot be due to lack of delicacy in measurement, for decided differences in thickness were recorded between different leaves, the readings running from 0.12 to 0.25 mm. Leaves on the same twig all tended to have the same thickness, but twigs differed among themselves, those from relatively shaded positions in the tree tending to be thinner than those which were more exposed to the sunlight. If light intensity determines blade thickness, as commonly supposed, we must infer that on a given twig the leaves in these four positions receive approximately the same degree of illumination.

Differences in Shape

The leaf groups also differ in shape. It will be noted that the members of the vertical pairs are symmetrical, the right and left main veins being equal. In the horizontal leaves, however, the lower or abaxial main lateral veins (right vein of the right leaf and left vein of the left) are distinctly longer than the upper ones, this difference being more than five times its probable error.

In the ratio of petiole length to blade (midrib) length the four groups also differ, as is shown in table 2. In the vertical lower leaves the petiole is far longer in proportion to the blade than it is in the vertical upper; and the members of the horizontal pairs are again intermediate.

Table 2. *Ratio of Blade Length to Petiole Length in the Four Leaf Groups*

	Blade Length / Petiole Length
Vertical upper	1.69
Vertical lower	.95
Horizontal right	1.11
Horizontal left	1.11

Finally, the four groups differ in blade shape, as shown by the width-to-length ratio in table 3. The vertical upper leaves are relatively short and broad, the vertical lower ones relatively long and narrow, and the horizontals intermediate.

Table 3. *Ratio of Blade Width to Blade Length in the Four Leaf Groups*

	Blade Width / Blade Length
Vertical upper	1.45
Vertical lower	1.29
Horizontal right	1.35
Horizontal left	1.33

Differences in Variability

A study of table 1 shows that some dimensions are much more variable than others,[1] petiole length having the highest coefficient of variability and blade thickness the lowest. For any given size character the four groups also differ in variability in many cases. In every instance (except blade thickness), the vertical upper leaves are much more variable than the horizontal ones, and the vertical lower are usually intermediate. Although the difference between the vertical upper and the horizontal leaves is not in every case significant in comparison with its probable error, the figures justify us in regarding the vertical upper leaves as the most variable of the four groups.

[1] The coefficients of variability for those size characters which involve two dimensions (blade area) or three dimensions (blade volume) are of course necessarily higher than, and thus not comparable with, those involving only one dimension.

Another difference in variability which is noteworthy is that between the length of the midrib and of the lateral ribs, especially in the horizontally oriented leaves. Here there is an average difference of 6.4 percent, which is more than four and a half times its probable error and thus evidently significant.

Other Evidence

Aside from these gross measurements, the authors examined the terminal buds of horizontal shoots during the winter and found (as has been noted by others) that the primordia of the four leaf groups were essentially equal in size, indicating that visible differentiation, at least, does not begin until the shoot starts to grow. The experiments of earlier workers on twig-reversal were also repeated and confirmed, it being found possible to reverse the anisophylly completely by twisting a horizontal stem through an angle of 180°, provided this was done before the buds opened.

Discussion

We should now consider the bearing of our results on the various theories which have been put forward to account for anisophylly.

The evidence with regard to light is significant. It is a matter of common observation that the thickness of the blade is generally directly proportional to the amount of light it receives during development. The fact that the four leaf groups are identical in blade thickness therefore justifies us in concluding that they must receive essentially the same amount of sunlight and that light cannot be a factor of very great importance in determining the marked differences which exist between them.

The case is different with regard to gravity, however. Not only are all the foliar structures on the lower half of the branch much larger than on the upper, but the shape of these organs is what we should expect to result if the downward pull of gravity is really effective in development; for the vertical upper leaves are "telescoped," the blade being relatively short in proportion to its width and the petiole relatively short in proportion to the blade; and the vertical lower leaves are extended or drawn out, the blade being relatively long in proportion to its width and the petiole relatively long in proportion to the blade.

It may be objected that these results are really due to some other factor and that neither these facts nor the experimental data gathered by ourselves and others at all prove that gravity is really the dominant factor concerned. In this connection the results as to relative variability are of interest. We have shown that the vertical upper leaves are the most variable of all in their size characters (the vertical lower ones probably ranking next), and that the lateral veins in the horizontal leaves are more variable in length than the midrib. This is difficult to explain until we study individual twigs and find that the intensity of the anisophylly which they display is

not uniform but varies somewhat from twig to twig. Furthermore, an examination of twigs growing in all orientations shows that anisophylly appears very slightly in those twigs which are but slightly inclined from the vertical and becomes more and more marked as we approach the horizontal. The upper leaf is therefore relatively smaller in proportion to the lower in some of our twigs than in others; and since we have shown that the amount of leaf material at a given node tends to be independent of the way in which this material is apportioned between the two leaves, it follows that these differences in relative size between upper and lower leaves will be much greater *in proportion to the size of the leaf* in the upper leaves than they are in the lower, and that the variability in size of the upper leaves will therefore be greater than that of the lower, the result which we actually find to exist. The horizontal leaves, being relatively unaffected as to their volume, area, petiole length, and midrib length by these slight differences, will tend to be the least variable of all. In these leaves, however, the difference in length between the upper and lower main lateral veins also varies with the degree of anisophylly and thus with the orientation of the twig, increasing as we approach the horizontal. In a group of twigs varying slightly in the degree of anisophylly which they show (as do ours), these lateral veins will therefore tend to be more variable in length than the midrib, as we found actually to be the case. These differences in degree of variability to which we have called attention are probably only the natural result of slight differences in the intensity of the anisophylly displayed, which in turn seem to be due to, or at least to parallel, differences in *the orientation of the twigs to the horizontal.* Although this does not prove that gravity is the major factor here operating, it certainly favors such a conclusion.

As to exotrophy and other internal factors which involve a relation between the twig and its mother-shoot and are believed by Wiesner and others to stimulate growth on the outer (thus in horizontal twigs the lower) side of the stem, our evidence is not of decisive value. Such a factor may be operative but it is evidently not constant in its effect, since we find that twigs vary in the degree of their anisophylly. It may be argued that the size of the angle between twig and mother-shoot may determine the intensity of anisophyllous development, but a study of individual branches does not confirm such a conclusion but rather suggests that absolute orientation to the horizontal is the chief factor.

Evidence from this biometrical study of anisophylly therefore suggests that gravity is the major factor in producing the phenomenon in Acer. Other factors, both external and internal, may perhaps play a part, and the only conclusive evidence as to their relative importance must be derived from carefully controlled experimental work.

Summary

1. In horizontal twigs of certain species there are marked differences between the leaves borne on the upper and those on the lower sides of the twig. Difference of opinion exists as to the causes of this anisophylly.

2. In horizontal anisophyllous twigs of a single tree of *Acer saccharum*, measurements were made of the various linear dimensions and of blade area and volume in 1000 leaves, these being divided into four groups according to their position on the twig—the upper and lower members of the vertically oriented pairs and the right and left members of the horizontally oriented pairs.

3. The vertical upper leaves are the smallest, the vertical lower the largest, and the horizontal leaves are intermediate between these. At a given node the combined size of the two members of a leaf pair is essentially the same regardless of whether the pair is a vertically or a horizontally oriented one.

4. In thickness of leaf-blade, the four groups are almost identical.

5. In proportion to their midrib length, the vertical upper leaves are the broadest and have the shortest petioles; the vertical lower leaves are the narrowest and have the longest petioles, and the two horizontal leaves are alike and intermediate between the two former. In each horizontal leaf the lower lateral vein is markedly longer than the upper.

6. The vertical upper leaves tend to be the most variable in their dimensions, the vertical lower next, and the horizontal leaves the least. In the horizontal leaves the two main lateral veins are more variable than the midrib. These differences in variability are evidently due to the fact that the twigs studied differ somewhat in the degree of anisophylly which they display. This is probably due, in turn, to slight differences in the orientation of the twigs with reference to the horizontal, since a study of the degree of anisophylly in this species indicates that its intensity is proportional to the degree to which the twig diverges from the vertical.

7. Evidence here presented indicates that gravity is more important than light or other environmental or internal factors in producing anisophylly in Acer.

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IS IT MALTOSE OR PHLORIDZIN THAT IS ABUNDANT IN APPLE TISSUE?

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A recent paper by Mitra[1] on the seasonal changes of certain carbohydrates in apple trees, records the presence of very considerable quantities of maltose (0.6–5 percent) and discusses its behavior in relation to temperature, acidity, and enzym activity. That maltose should be so abundant in apple tissue is, alone, a point of great interest, and, adding thereto Mitra's data on seasonal fluctuations of this sugar and his explanations of such fluctuations, the whole constitutes a very important contribution to our knowledge of the physiology of the apple tree, should his findings be substantiated. Such striking conclusions as the following, quoted from Mitra's publication, would seem to deserve considerable attention:

> The translocation of sugars in apple spurs is largely in the form of glucose and maltose. . . . Maltose appears to be the most important sugar of translocation during starch accumulation in autumn. To sum up, the upward translocation of sugar in spring is largely in the form of glucose, while translocation of sugar from leaves to stem in autumn is largely in the form of maltose. . . . Maltose is most abundant and glucose is least abundant in summer and autumn, when acidity is highest and nearer the optimum for diastase than for maltase. On the other hand, maltose is lowest and glucose highest during the dormant period, when acidity is lowest and nearer the optimum for maltase than for diastase. . . . These facts suggest an explanation of why maltose is the most important sugar of translocation from leaves to stem in summer.

The significance of the above-quoted conclusions for plant physiology and theoretical horticulture is apparent and justifies a rather close scrutiny into the methods and material from which they are derived. The present paper is an examination of the maltose situation in the apple as reported by Mitra, and presents reasons for a reconsideration of his data respecting it.

The large figures reported by Mitra for maltose led the writer to an examination of the method and conditions under which his analytical data for maltose were probably obtained. At the outset, however, this critical attitude arose largely through the writer's own experience of several years in the analysis of apple tissue, although many of the statements to follow are based on subsequent specific study of the question.

The method employed by Mitra for maltose was that described by

[1] Mitra, S. K. Seasonal changes and translocation of carbohydrate materials in fruit spurs and two-year-old seedlings of apple. Ohio Jour. Sci. **21**: 89–103. 1921.

Darwin and Acton,[2] and is based on the fact that maltose is not hydrolyzed by the regular sucrose-inversion procedure, but requires a more severe acid treatment, so that, after regular inversion, the solution is subjected to another hydrolysis with 2-percent hydrochloric acid for three hours. The increased reducing power of the solution after this treatment is assumed to be due to the splitting up of maltose. This latter hydrolytic treatment has been called "complete inversion," and as such it will be referred to throughout this paper. The increased reducing power after "complete inversion" is multiplied by the factor 2.32 to give the quantity of maltose originally present. The method has received severe criticism from David and Daish,[3] but the writer immediately questioned the use of this method in the analysis of apple tissue for an additional reason, namely, that the bark of all parts of an apple tree contains considerable quantities of the glucoside phloridzin, which might be hydrolyzed by the "complete-inversion" treatment. If so, glucose from phloridzin would then increase the reducing power of the solution as though it were from maltose. Since it was suspected that the quantity of phloridzin might be as much as 1 or 2 percent in apple tissue, any figures for maltose based on this method seemed open to serious criticism.

Toward an understanding of the relations between maltose and phloridzin in apple tissue, the following considerations and tests were made:

Qualitative Considerations. In chemical literature phloridzin has frequently been reported as a normal constituent of apple tissue, and the writer himself has repeatedly isolated and prepared pure crystalline phloridzin from spurs and shoots of apple trees. It is always possible to obtain a positive test for phloridzin in apple bark by means of such reagents as ferric chloride, mercuric nitrate, or strong sulphuric acid, while it is quite impossible to secure a positive test for maltose in similar tissue by such reagents as phenylhydrazine.

Behavior of Pure Phloridzin under the "Complete-Inversion" Treatment. To determine the extent to which phloridzin is affected by the methods of sugar analysis, 2.426 grams of crystalline phloridzin (equivalent to 2.24 grams anhydrous phloridzin) were dissolved in water, and the volume was made up to 1000 cubic centimeters. Portions were carried through the regular procedures for (*a*) "direct-reducing" sugars; (*b*) "total sugars after inversion"; and (*c*) "complete inversion." In each case the amount of solution submitted to the reduction procedure contained 0.083 gram of anhydrous phloridzin, which on complete hydrolysis should yield 34.2 milligrams of glucose, according to the accepted formula for phloridzin:

$$\underset{\text{Phloridzin}}{C_{21}H_{24}O_{10}} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Phloretin}}{C_{15}H_{14}O_5}$$

[2] Darwin and Acton. Practical physiology of plants, p. 285. 1901 (reprint, 1909).

[3] David, W. A., and Daish, A. J. A study of the methods of estimation of carbohydrates, especially in plant extracts. Jour. Agr. Sci. 5: 437–468. 1913.

The average amount of glucose found after each of the three procedures was: for (*a*) none; for (*b*) trace (?); and for (*c*) 33.3 mg. respectively. This indicates that phloridzin does not reduce copper either in the direct reduction or after inversion procedures, but that it is quantitatively hydrolyzed by the "complete-inversion" treatment.

Relative Behavior of Maltose and Phloridzin with Mixtures of other Sugars. Two definite mixtures of pure chemicals were made up: one contained maltose with glucose and sucrose; the other, phloridzin with glucose and sucrose. Analyses were run on each for directly reducing sugars, total sugars after inversion, and those after complete inversion. It was found that in these mixtures all the sugars and phloridzin could be recovered quantitatively by the regular procedure of sugar analysis, providing, of course, allowance was made for some destruction of glucose and fructose during the severe "complete-inversion" treatment.[4] Also, it should be kept in mind that the increased reducing power after complete inversion is multiplied by 2.42 in calculating phloridzin, and by 2.32 [5] in calculating maltose. It seems apparent from the results of these analyses that the method suggested by Darwin and Acton is quite as applicable for the quantitative determination of phloridzin as for that of maltose.

Application of the Darwin and Acton Method to the Analysis of Apple Tissue. The maltose method described was temporarily made an integral part of the analytical procedure in the analysis of 48 samples of summer shoots of apple, collected in connection with the regular horticultural experiments of the Station. These samples were of two varieties, Spitzenburg and Grimes, and had been collected at intervals from June 24 to August 29.

The increases in reducing power after "complete inversion" are presented in table 1, calculated both as phloridzin and as maltose. The values on each date are averages of duplicate determinations on four

TABLE 1. *Percentages of Maltose or Phloridzin in Apple Shoots*

Date of Collection	Increased Reducing Power Calculated as:		Maltose (as Reported by Mitra)
	Phloridzin	Maltose	
June 24	4.34	3.77	2.63
July 14	2.91	2.53	2.98
August 29	2.82	2.45	2.37

[4] The solutions subjected to the complete inversion contained a total of about 1 percent glucose and fructose after hydrolysis. Under these conditions, the amount destroyed by acid could safely be considered less than 4 percent of the total, although in one instance 7.8 percent was believed to have thus disappeared.

[5] The factor 2.1 seems to conform more closely with the reducing power of maltose under the conditions of analysis prescribed by the American A. O. A. C.

different lots of control, or untreated, summer shoots. In the table are included also the percentages of maltose reported by Mitra for tops of two-year-old apple seedlings on approximately corresponding dates. There is a striking similarity between the writer's maltose values and those of Mitra.

If it can be shown that the writer's values are really for phloridzin and not for maltose, there should be some hesitancy in accepting Mitra's values as maltose.

That the former figures are due to phloridzin is made quite clear by the two experiments reported below.

Estimation of Phloridzin by Means of its Product, Phloretin. In order to ascertain the exact contribution of maltose to the increased reducing power of apple extracts after "complete inversion," a method was devised for determining phloridzin by means of its hydrolytic product phloretin,[6] instead of by its glucose. A sample of apple extract after complete inversion yielded 0.148 gram of phloretin, which should correspond to 0.2356 gram of anhydrous phloridzin. This quantity of phloridzin should theoretically yield 0.0972 gram of glucose. On the same extract the increased reducing power was also determined and calculated as glucose. The glucose found was 0.0932 gram, a quantity which is strikingly equivalent to the phloretin found by the other method. The amount of phloridzin present as estimated by its phloretin and its glucose products were 2.36 and 2.27 percent respectively. The amount of maltose in the apple tissue examined must therefore be extremely small.

Estimation of Maltose by Fermentation with the Yeast, Saccharomyces marxianus. Finally, in order to be more certain regarding the question of occurrence of maltose in apple tissue, examination was made for maltose by the method suggested by David and Daish.[7] These authors found that maltose could be quantitatively determined most accurately in plant extracts by the aid of special yeasts, which are unable to ferment maltose but readily ferment hexoses, sucrose, and other lower hexose derivatives. Of such yeasts there are several species, but from numerous tests they were able to designate three species as especially useful: namely, *Saccharomyces marxianus*, *S. anomalus*, and *S. exiguus*. After plant extracts have been submitted to fermentation by these yeasts, the residual sugars are assumed to be maltose and pentoses. Differentiation is then made between pentoses and maltose by a further fermentation with ordinary baker's yeast which leaves the pentoses only.

[6] The new method is extremely simple as here employed. The apple extract is carried through the regular procedures of analysis, including the complete-inversion treatment. Upon cooling and neutralizing after complete inversion, the phloretin separates quantitatively as an amorphous white precipitate which is readily filtered on a Gooch crucible, dried in a vacuum oven at 95–100° C., and weighed.

[7] *Loc. cit.*

In the present experiment some samples of apple extract were submitted to the fermentative action of *S. marxianus*.[8] This yeast grew very well in the extracts after a slight adjustment of acidity. A period of 45 days at 26 to 30° C. was allowed for complete fermentation. Examination of the cultures then showed that very little reducing substance had escaped fermentation. Later, when the residual reducing power of such cultures was compared with that of other cultures which had been fermented with baker's yeast, the values were so similar as to warrant the assumption that the residual reducing power of the former was due to free pentoses only, and that maltose was, from the quantitative aspect, a negligible quantity.

Summary and Conclusion

The present paper calls attention to a possible misinterpretation of analytical data by Mitra; namely, that the increased reducing power of an apple extract after complete inversion is due to maltose, while the writer believes it to be due to the hydrolysis of phloridzin. In support of this latter viewpoint, the paper has presented the following points for consideration:

1. Phloridzin is a normal constituent of apple bark and maltose has not been shown to be.

2. Pure phloridzin is completely hydrolyzed to glucose and phloretin under the conditions of the Darwin and Acton method used by Mitra.

3. Phloridzin in mixtures of sugars behaves similarly to maltose, and can be quite as accurately determined by the Darwin and Acton method.

4. This method when applied to apple tissue gave values which, when interpreted as either phloridzin or maltose, agreed closely with those reported by Mitra, using similar methods and material.

5. The direct determination of phloretin in apple tissue gave phloridzin percentages comparable with those obtained by means of glucose (*i.e.*, by the method of Darwin and Acton). Thus it was shown that the increased reducing power of apple tissue after a "complete inversion" is due to phloridzin and not to maltose.

6. An application of the fermentation method of David and Daish failed to show the presence of detectable quantities of maltose.

In conclusion, the writer wishes to repeat that these statements simply call for a reconsideration of the maltose values reported by Mitra and do not necessarily show that *his* material did not contain maltose, seeing that his was younger, probably of a different variety, and from a different

[8] The culture of *Saccharomyes marxianus* was procured through the kindness and coöperation of Professor G. V. Copson of the Department of Bacteriology. In cooperation with Professor Copson, the culture was thoroughly tested as to its fermentation characteristics and found to behave normally, that is to say, it readily fermented all the common sugars except maltose. Later the Bacteriological Department aided in the cultivation of the yeast in the extracts of apple tissue.

section of the country. But for the present, in this laboratory, such maltose percentages are multiplied by a factor of about 1.04 and interpreted as chiefly phloridzin.

Finally, it may be added, if all maltose values reported for apple tissue are really of phloridzin, then the function of maltose and the explanation of its behavior, as outlined by Mitra, can scarcely longer be accepted. Nevertheless, there would remain the very interesting question: What, then, is the meaning of this remarkable seasonal behavior of *phloridzin?*

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CRYPTOMORPHA, A NEW SECTION OF SAXIFRAGA

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While pursuing some studies on the section Boraphila Engler, of the genus Saxifraga, the writer (4) found that the flower of *Saxifraga eriophora* S. Watson showed some important differences in structure as compared with the other species of this section. With better material at hand, kindly loaned by the Herbarium of the University of California, it has been possible to make a more thorough analysis of the flower of this species, with the result that the writer here undertakes to make it the basis of a new section.

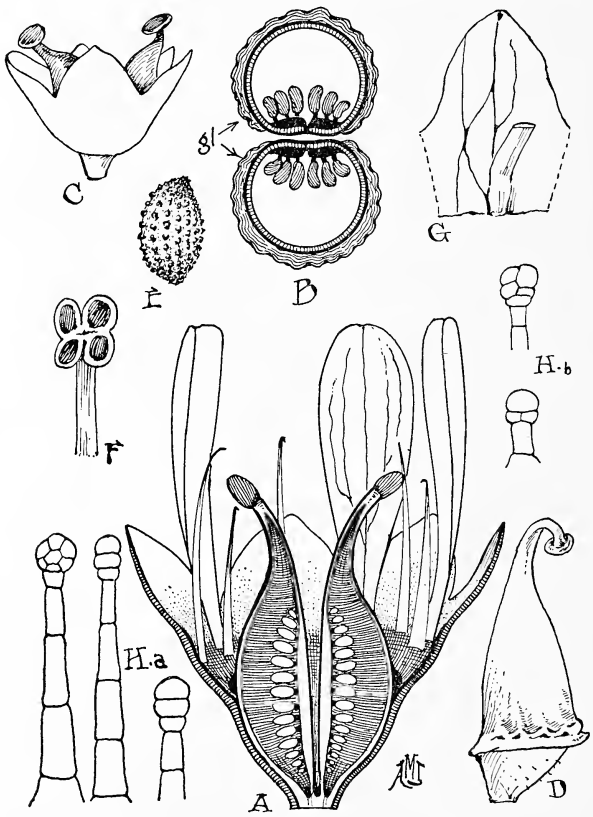

FIG. 1. *A*, Semidiagrammatic vertical section of the flower of *Saxifraga eriophora* S. Watson. *B*, Cross-section of the ovaries *in situ*, in the region of the gland, showing placentation and ovules, and glands surrounding ovaries; *gl*, gland. *C*, Semidiagrammatic aspect of a young flower, the stamens and petals removed. *D*, Lateral aspect of a single carpel, with parts of the flower cut away to show the gland. *E*, A mature seed. *F*, An anther, some time after shedding of pollen. *G*, A single segment of the calyx with part of the calyx-tube (hypanthium), showing nervation and the insertion of a stamen. *H.a* and *H.b*, Various types of hairs.

Watson (1) regarded *Saxifraga eriophora* S. Watson as closely related to *Saxifraga virginiensis* Michx. and *S. nivalis* L. It is true that these three species are very similar in vegetative characters, but, as will be shown below, the flower of *Saxifraga eriophora* S. Wats. is distinctly different in structure from the flowers of the other two above-named species. In like manner, Engler (2) placed the species with *Saxifraga virginiensis* S. Wats., *S. nivalis* L., and *S. reflexa* Hook., in a group of the section Boraphila characterized by a compact inflorescence ("Blütenstand dicht gedrängt"). Later, Small and Rydberg (3) included the species in the genus Micranthes Haw.

The important facts about the flower of *Saxifraga eriophora* S. Wats. are that the ovaries of the two otherwise distinct carpels are consolidated with the receptacle for nearly half their length, that is, the ovaries are half-inferior, the receptacle being further prolonged and expanded into a rather deep calyx-tube (hypanthium) in the middle region of which the stamens are inserted. As in many other species of Saxifraga, the ovaries are surrounded dorsally and laterally by a sinuous disk or gland, which in this case persists at maturity as a dry flange a short distance above the base of the hypanthium.

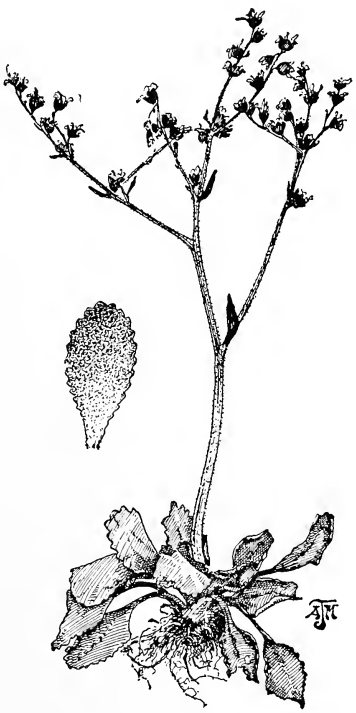

FIG. 2. *Saxifraga eriophora* S. Watson. Drawn from a specimen in the Herbarium of the University of California. Height of scape, 15 cm. At the left is a sketch of the lower surface of a leaf-blade, showing tomentum.

CRYPTOMORPHA sect. nov.

Carpella 2 elongata distincta in divergentes stilos attenuatos contracta ovariis semiinferis glandula supra hypanthii basim cinctis staminibus in hypanthii parte superiore instructis.

Carpels two, elongate, distinct, contracted into slender divergent styles; ovaries semi-inferior, surrounded by a gland above the base of the hypanthium; stamens inserted on the upper part of the hypanthium.

Saxifraga eriophora S. Watson, Proc. Amer. Acad. **17**: 372. 1881–1882.
Micranthes eriophora (S. Wats.) Small, N. Amer. Fl. **22**[2]: 142. 1905.

Scape erect, rigid, 15–18 cm. high, sparsely and rather obscurely rufous-pubescent, cymosely branched from the middle, the branches spreading. Leaves basal, 2–4 cm. long, ovate to elliptic-ovate, more or less abruptly contracted into the petioles, glabrous above, more or less densely rufous-tomentose beneath. Flowers small, numerous; pedicels shorter than the flowers, subtended by small lanceolate bracts. Hairs multicellular, consisting of a single row of cells, the glands multicellular, oblong or globose, when oblong consisting of a single series of superposed cells. Sepals deltoid or triangular-oblong, ascending-spreading, sparsely glandular-pubescent dorsally. Petals spatulate-oblong, gradually contracted toward the base, about twice as long as the sepals, inserted on the hypanthium. Stamens 10, filaments plano-subulate, broadest downwards, strongly contracted and incurving above; anthers small. Follicles small, erect; stigmas oblong or nearly globose. Seeds 0.3 mm. long, elliptic to elliptic-ovoid, with longitudinal lines of short, blunt, tuberculate teeth. Perennial from a short, stout, erect caudex. Roots fibrous.

Distribution. Known only from the Santa Catalina mountains of Arizona, where it is reported to be common.

Specimens examined. Ex Lemmon Herbarium, Oakland, California, "N. slope Santa Catalina Mts., 8000 ft. alt., May 1, 1881. Flora of Arizona." Collected by J. C. Lemmon and wife. Missouri Botanical Garden no. *84214*, and Herbarium of the University of California, no. *114893*.

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THE BOLIVIAN SPECIES OF VERNONIA

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Rapid progress has been made in extending knowledge of the flora of Bolivia during the past quarter-century. Bang and Buchtien, stationed in the country, have distributed numerous specimens, several European botanists have collected in it, particularly along its southern boundary, and the expeditions of Rusby, Williams, and White have been especially productive. While many parts of its great extent are practically unknown botanically and will doubtless yield many unreported species, it may be desirable to summarize the species of Vernonia so far as they are at present represented at the New York Botanical Garden.

This vast genus is greatly developed in southern Brazil, Paraguay, and Uruguay, where it forms one of the most important components of the flora. These areas have mostly been approached from the east, and it is not known how many of their species extend westward into Bolivia. Another center of development is along the Andean chain of mountains, from Colombia to Bolivia. Since the mountainous portion of Bolivia has been more thoroughly botanized than the eastern portion of the country, the species of this general distribution and relationship are better known.

Lying at the western limits of the Vernoniae of the Brazilian center, and at the southern extremity of those of the Andean region, Bolivia exhibits only the end results of evolution, and the species give little clue accordingly to their mutual relationships within the genus. The following arrangement of species may need considerable revision after careful study has been made of the probable evolution of the Brazilian species.

The three sections Stenocephalum, Critoniopsis, and Lepidaploa have been distinguished on the usual morphological characters. Within the last-named section, groups have been segregated according to the inflorescence, since experience with North American species has indicated that this character may be of great evolutionary importance.

Thanks are due to Dr. William R. Maxon for the loan of material from the Buchtien Herbarium, recently acquired by the National Museum.

Involucre cylindric, few-flowered, its scales closely imbricate, permanently erect, coriaceous, subulate or spinose at the tip; inflorescence spicate. — Section 1. STENOCEPHALUM.

Involucres small, of few but imbricate scales, the inner deciduous at maturity, the outer eventually spreading, or permanently erect; pappus fragile, the outer series poorly differentiated; inflorescence a widely and freely branched compound cyme. — Section 2. CRITONIOPSIS.

Involucres small to large, few- to many-flowered, the scales persistent, prominently spreading or reflexed at maturity; achenes ribbed; pappus mostly in two distinct series. Section 3. LEPIDAPLOA.

All or most of the heads subtended by foliaceous bracteal leaves, sessile or nearly so. Subsection 3*a*.

Heads subtended by minute scale-like bracteal leaves or none, sessile or pediceled.

Cymes long, divergent, straight or curved, but maintaining a considerable distance without branches; heads crowded, sessile. Subsection 3*b*.

Cymes reduced to 1 or a few heads, which are sessile and terminal or on long terminal or subterminal peduncles; heads mostly large. Subsection 3*c*.

Cymes abbreviated, the heads in capitulate clusters, sessile; involucre funnel-form, bearing numerous scales on its narrowed base. Subsection 3*d*.

Cymes freely branched, the branches appearing at the bases of many of the sessile heads and producing a large paniculate cluster. Subsection 3*e*.

Cymes freely branched and greatly reduced, mostly bearing 1 or 2 heads only, which therefore appear peduncled. Subsection 3*f*.

Section 1. STENOCEPHALUM

1. *V. apiculata* Mart., DC. Prodr. **5**: 51. 1836.
Oripati, Yungas, Bang *2166;* Apolo, Williams *20,123*.

Section 2. CRITONIOPSIS

Leaves sessile and clasping by a broad auriculate base, broadest at or near the middle, densely pubescent or tomentose beneath, the larger ones 15–20 cm. long; inflorescence very large and open; heads about 8-flowered; involucre obconic, about 4 mm. high, straw-colored or brown; scales obtuse or rounded; achenes thinly pubescent in 5 lines; pappus white, the deciduous bristles 4 mm. long, the paleae 1 mm. long, scarcely wider than the bristles.

Stem, branches, inflorescence, and lower leaf-surface densely shaggy with gray-brown hairs 3–5 mm. long; leaves broadly oblong-elliptic; involucre hairy toward the base, becoming glabrate toward the summit. 2. *V. jubifera.*

Stem, branches, inflorescence, and lower leaf-surface densely appressed-pubescent or subtomentose; leaves ovate-oblong; involucral scales thinly villous or glabrate toward the tip. 3. *V. Conwayi.*

Leaves petioled, 10–15 cm. long; involucre 3 mm. high; scales rounded to obtuse or acute, mostly tomentose at the tip; pappus gray or tawny.

Leaves and stem essentially glabrous; leaves acuminate; heads 5-flowered; achenes very thinly hirsute. 4. *V. boliviana.*

Leaves and stem pubescent or tomentose; heads 5- –8-flowered; achenes nearly or quite glabrous.

Leaves softly ferruginous-tomentose beneath, acuminate at the base, acute or subacuminate at apex, entire. 5. *V. pycnantha.*

Leaves tomentose beneath only on the prominently reticulate veins, truncate or subcordate at base, acuminate at apex, minutely spinose-denticulate. 6. *V. yungasensis.*

2. *V. jubifera* Rusby, Mem. Torrey Bot. Club **6**: 53. 1896.
Between Mapiri and Tipuani, Bang *1554* (type).

3. *V. Conwayi* Rusby, Bull. N. Y. Bot. Gard. **8**: 125. 1912.
Near Inglis-Inglis, Williams *1493* (type).

4. *V. boliviana* Britton, Bull. Torrey Bot. Club **18**: 332. 1891.
Near Yungas, Rusby *1729* (type); Coroico, Yungas, Bang *2409;* without further data, Bang.

5. *V. pycnantha* Benth., Pl. Hartweg. 134. 1844.
Yungas, Rusby *1728*.

6. *V. yungasensis* Britton, Bull. Torrey Bot. Club **18**: 332. 1891.
Near Yungas, Rusby *1731*, Rusby *1732* (type).

Section 3. Lepidaploa

Subsection 3*a*

Bracts resembling the leaves in shape, and but little if any reduced in size.

Leaves linear.

Heads small, the involucre 6–7 mm. high; leaves somewhat revolute, narrowly linear, 2–3 mm. wide, the lateral veins suppressed. 7. *V. rubricaulis.*

Heads large, the involucre 10–16 mm. high; leaves thick and heavy, broadly linear, 5–15 mm. wide, the lateral veins prominent.

Outer involucral scales broadly ovate, subacute and mucronulate; involucre about 11 mm. high; leaves closely tomentose beneath. 8. *V. ligulaefolia.*

Outer involucral scales lanceolate, long-acuminate or subulate, involucre about 16 mm. high; leaves thinly pubescent beneath. 9. *V. ixiamensis.*

Leaves broader than linear.

Involucre 6 mm. high. 10. *V. saltensis.*

Involucre 10–13 mm. high, broadly campanulate to hemispheric.

Middle scales subacute or obtuse, closely appressed, almost glabrous, the inner purple and prominently puberulent with short yellowish hairs. 11. *V. varroniaefolia.*

Middle scales sharply acute to subulate, usually spreading. 12. *V. robusta.*

Bracts much smaller than the leaves.

Involucre narrowly campanulate or subcylindric, its scales obtuse or subacute, appressed, closely imbricate; heads about 8-flowered; achenes hirsute on the ribs only. 13. *V. obtusata.*

Involucre broadly obconic, its scales long-acuminate or subulate, imbricate at the base, loosely erect or somewhat spreading above.
Leaves of an ovate type, about twice as long as broad, softly pubescent above, tomentose beneath, the lateral veins prominent, curved-ascending and parallel. 14. *V. Mandonii.*
Leaves of an oblong type, the lateral veins not prominent or parallel.
Leaves very minutely puberulent beneath; involucral scales minutely strigose, appearing almost glabrous; involucre 9–12 mm. high. 15. *V. tarijensis.*
Leaves hirsute beneath; involucre 6–7 mm. high, its scales sericeous or hirsute.
Stem densely pubescent or subtomentose with purplish hairs. 16. *V. cuneifolia.*
Stem thinly pubescent.
Leaves densely pubescent beneath and not resinous; pappus brown or straw-colored. 17. *V. remotiflora.*
Leaves strigose-pubescent and resinous beneath; pappus white. 18. *V. argyropappa.*
Leaves linear, lanceolate, or narrowly oblong; pappus dull brown.
Leaves rounded, truncate, or subcordate at base, the pubescence of the lower surface strigose, on the surface and veins alike. 19. *V. aristosquamosa.*
Leaves acuminate at base, the pubescence of the lower surface all or chiefly on the veins.
Scales purple; leaves comose on the veins beneath. 20. *V. Buchtieni.*
Scales straw-colored; leaves thinly pilose on the veins beneath. 21. *V. deflexa.*

7. *V. rubricaulis* HBK., Nov. Gen. & Sp. 4: 33. 1818.

Several varieties of this widely distributed species have been recognized by Hieronymus in adjacent parts of South America. It seems to be commoner east of Bolivia, from which few specimens have as yet been collected.

Velasco, Kuntze; without precise locality, White *1520*.

8. *V. ligulaefolia* Mart., DC. Prodr. 5: 45. 1836.

The single sheet examined, Williams *90*, from Apolo, was questionably referred to this species by Ekman. It agrees well with the original description, except in the size of the leaves, but only the upper ones are represented.

9. *V. ixiamensis* Rusby, Bull. N. Y. Bot. Gard. 8: 125. 1912.

This striking and well-marked species has recently been recollected at or near the type locality.

Ixiamos, Williams *284* (type), Cardanos *2017*.

10. *V. saltensis* Hieron., Engler's Jahrb. 22: 691. 1897.

The detailed original description applies perfectly to the single Bolivian specimen examined, Fiebrig *2741*, from Chiquiaca.

11. *V. varroniaefolia* DC., Prodr. **5**: 56. 1836.

The single collection examined, Bang *2218*, without locality, has been provisionally referred here. Ekman has noted that it differs from the type in its larger heads, but otherwise it agrees well with the original description, and even better with that in the Flora Brasiliensis.

12. *V. robusta* Rusby, Mem. Torrey Bot. Club. **6**: 54. 1896.

The material examined is sufficient to show that the species varies considerably in form of leaf and in character of involucre, without presenting any features on which species may be segregated.

Between Guanai and Tipuani, Bang *1424* (type); Atten, Williams *1441;* Coripati, Yungas, Bang *2118;* Velasco, Kuntze; without locality, Bang *2886*.

Another sheet collected at Velasco by Kuntze has been annotated as "nicht beschreibbar" by Hieronymus, but Ekman has given it the unpublished name *V. velascensis* Hieron. It is fragmentary, lacking foliage leaves completely. The involucre is more nearly glabrous than in typical *V. robusta*, and the outer scales are much more pungent and distinctly squarrose. It is now referred provisionally to this species.

13. *V. obtusata* Less., Linnaea **6**: 62. 1831.

Velasco, Kuntze.

14. *V. Mandonii* Sch.-Bip., Linnaea **34**: 534. 1865–66. Name only.

The species is beautifully characterized by its broadly ovate leaves with prominent parallel veins. No description of it has been found, and it is possible that the characterization presented here in the key to the species constitutes its first valid publication.

Near Sorata, province of Larecaja, Mandon *234* (isotype).

15. *V. tarijensis* (Griseb.) Hieron., Engler's Jahrb. **22**: 682. 1897.

V. sericea tarijensis Griseb., Goett. Abhandl. **24**: 163. 1879.

Distinguished at once from the related species by the glabrate leaves and large heads.

Sierra de Santa Cruz, Kuntze; Velasco, Kuntze.

16. **V. cuneifolia** (Britton) n. sp.

V. arborescens cuneifolia Britton, Bull. Torrey Bot. Club **18**: 331. 1891.

Stem stout, densely pubescent with short, stout, spreading, purplish hairs; leaves broadly oblong-elliptic to obovate, up to 8 cm. long by 32 mm. wide, abruptly acute or very short acuminate, entire, cuneate or acuminate at base into a short indefinite petiole, thinly pubescent above with papillose hairs, becoming glabrate and scabrous with the persistent hair-bases, thinly pubescent beneath with rather long papillose hairs, the veins prominent and more or less reticulate, impressed above; inflorescence dense, freely branched, of numerous short irregular cymes; heads about 26-flowered, numerous, irregularly spaced, up to 2 cm. apart or some approximate; bracts narrow, much reduced, the upper about equaling the heads; involucre broadly obconic, 6 mm. high, the scales appressed below, loosely erect above, brown or faintly purple-tinged, narrowly lanceolate, long-acute or subacuminate,

densely sericeous with erect hairs; achenes thinly hirsute; pappus bristles almost white, 6 mm. long, the paleae slender, scarcely wider than the bristles, 1.5 mm. long.

Reis, Rusby *2148*.

17. *V. remotiflora* L. C. Rich., Act. Soc. Hist. Nat. Paris **1**: 112. 1792.
Santa Cruz, Kuntze; Velasco, Kuntze.

18. *V. argyropappa* Buek, Index Prodr. **2**: v. 1840.
Without locality, Bang *1428*.

19. *V. aristosquamosa* Britton, Bull. Torrey Bot. Club **18**: 332. 1891.
Yungas, Rusby *1657* (type); Apolo, Williams *1496;* Atten, Williams *1443;* San Carlos, near Mapiri, Buchtien *1529*.

20. **V. Buchtieni** n. sp.

Stems suffruticose, erect, sparingly branched, 3–4 dm. high, softly pubescent above, becoming thinly pubescent or glabrate with age; leaves rather crowded, the blades linear-oblong, broadest near the middle, 5–6 cm. long, 6–11 mm. wide, acute, entire, gradually acuminate or cuneate to the base, sessile or on petioles 2–3 mm. long, thinly pubescent with papillose hairs or becoming glabrate above, comose beneath with brownish hairs on the midvein and margin and sparingly hirsute on the surface, the midvein prominent beneath, the lateral veins faint, sharply ascending and irregular; cymes 2–4, terminal and subterminal, scarcely branched, as much as 8 cm. long, bearing 2–6 heads; bracts resembling the leaves but smaller, the lower 25 mm. long, the upper gradually reduced to 10 mm. long; heads sessile, about 21-flowered; involucre campanulate, 7 mm. high, dull purple; scales appressed below, loosely erect or somewhat spreading above, narrowly lanceolate, long-acuminate, nearly glabrous; achenes thinly hirsute, 3 mm. long; pappus dull brown, the bristles 6 mm. long, the outer scales broadly linear, 0.9–1.1 mm. long.

Type: Buchtien *1528*, collected near Mapiri, at an altitude of 700 meters, and deposited in the herbarium of the New York Botanical Garden.

V. Buchtieni is closely related to *V. deflexa* Rusby, from which it differs in the characteristic pubescence of the leaves and the color of the involucre.

21. *V. deflexa* Rusby, Bull. N. Y. Bot. Gard. **4**: 376. 1907.
Atten, Williams *1444*.

Subsection *3b*

Leaves oblanceolate, the principal ones 2 dm. or more long, mostly sharply toothed; inflorescence very large; involucral scales subacute or obtuse. 22. *V. megaphylla.*

Leaves ovate or lanceolate, the principal ones not exceeding 1 dm. in length; inflorescence medium in size; involucral scales acuminate to subulate. 23. *V. scorpioides.*

22. *V. megaphylla* Hieron., Verhandl. Bot. Ver. Brandenburg **48**: 195. 1907.
V. digitata Rusby, Bull. N. Y. Bot. Gard. **8**: 125. 1912.
The huge leaves and unusually long straight cymes distinguish this

species at once. It should be noted that Hieronymus' statement that the leaves are 1.5 cm. wide is probably a misprint for 1.5 dm.

Mapiri, Buchtien *1527;* above Corendo, White *911;* cataracts of the Bopi River, Rusby *488;* Mapiri, Williams *713* (type of *V. digitata*).

23. *V. scorpioides* (Lam.) Pers., Syn. **2**: 404. 1807.

V. cincta Griseb., Goett. Abhandl. **24**: 162. 1879.

V. breviramosa Rusby, Bull. N. Y. Bot. Gard. **8**: 123. 1912.

Material identified as *V. cincta* Griseb. by Hieronymus can not be separated specifically from this common and widely distributed species.

Apolo, Williams *1431* (type of *V. breviramosa*); Santa Cruz, Kuntze; Velasco, Kuntze; Yapacani, Kuntze; Coroico, North Yungas, Buchtien *432, 3928;* North Yungas, Buchtien *4746;* South Yungas, Buchtien *277;* Yungas, Rusby *1704,* Bang *405, 222;* Antahuacana, Buchtien *4747;* Canamina, Rusby *47;* Tunari, Kuntze; Charopampa, Williams *769.*

Subsection 3*c*

Involucral scales lance-subulate; involucre barely exceeding 10 mm. in height, the common peduncle prolonged 1–2 dm. above the leaves. 24. *V. simplex.*

Involucral scales broad and obtuse; involucre mostly about 15 mm. high.

Heads solitary or rarely 2 or 3; leaves narrowed to the base, not softly tomentose beneath; involucral scales nearly glabrous.

Leaves linear, long-acuminate, minutely puberulent and conspicuously glandular beneath. 25. *V. grandiflora.*

Leaves oblanceolate, obtuse or barely acute, prominently reticulate-veined, closely gray-tomentulose beneath. 26. *V. coriacea.*

Heads several, closely glomerate; leaves rounded at the base, oblong-lanceolate, densely and softly ferruginous-tomentose beneath; involucral scales tomentose. 27. *V. Kuntzei.*

24. *V. simplex* Less., Linnaea **4**: 280. 1829.

Ixiamos, Williams *282;* Sorata, Rusby *2669.*

25. *V. grandiflora* Less., Linnaea **6**: 660. 1831.

Chrysocoma pumila Vell., Fl. Flum. **8**: 331. 1825. Not *Vernonia pumila* Kotschy & Peyr. 1867.

Velasco, Kuntze.

26. *V. coriacea* Less., Linnaea **6**: 661. 1831.

Apolo, Williams *134;* Reis, Rusby *1588;* without locality, Bang *2888.*

27. *V. Kuntzei* Hieron., Engler's Jahrb. **22**: 678. 1897.

This well-marked species differs notably from the others of the subsection in its glomerate heads, and is referred here with considerable hesitation.

Santa Cruz, Kuntze (isotype).

Subsection 3*d*

28. *V. squamulosa* Hook. & Arn., in Hook. Comp. Bot. Mag. **2**: 44. 1836.

A singular species, strongly reminiscent of the group Buxifoliae of Hispaniola in its general habit.

Sierra de Santa Cruz, Kuntze.

Subsection 3*e*

Corolla-lobes strongly pubescent within; achenes densely resinous. 29. *V. echitifolia.*

Corolla-lobes glabrous within; achenes pubescent.

Middle and outer involucral scales flat, acuminate to rounded but never subulate, veinless or with an inconspicuous midvein.

Panicle raised on a long, leafless peduncle; leaves obovate, softly and densely tomentose beneath. 30. *V. herbacea.*

Panicle leafy at the base.

Leaves rounded or cordate at base, broadly ovate or oblong, thick, heavy, scabrous above.

Leaves distinctly cordate or subauriculate at base, closely short-pubescent beneath; exposed portion of the glabrate involucral scales broadly ovate. 31. *V. santacruzensis.*

Leaves rounded at base; exposed portion of the pubescent or arachnoid scales lanceolate or narrowly ovate.

Lower leaf-surface and inflorescence densely ferruginous-tomentose; involucral scales green, pubescent on the back chiefly near the apex. 32. *V. ferruginea.*

Lower leaf-surface pubescent or subsericeous with papillose hairs which are later deciduous; inflorescence densely pubescent with short ascending hairs; involucral scales purple at the tip, pubescent chiefly at the margin and base. 33. *V. crassifolia.*

Leaves narrowed to the acute or obtuse base.

Leaves obtuse or rounded, mostly prominently serrate.

Exposed portion of the principal involucral scales broadly ovate, obtuse or rounded and minutely apiculate; leaves narrowly oblong, glabrate beneath or minutely pubescent with short conic hairs. 34. *V. membranacea.*

Exposed portion of the principal scales broadly lanceolate, sharply acute; leaves broadly oblong to obovate, densely pubescent or subtomentose with crooked hairs beneath. 35. *V. brasiliana.*

Leaves acuminate, entire or obscurely serrate.

Pappus white or pale straw-colored.

Leaves glabrate beneath or minutely pubescent with short conic hairs. 36. *V. myriocephala.*

Leaves appressed-pubescent beneath with long papillose hairs. 37. *V. Lehmanni.*

Pappus tawny.

Middle and outer involucral scales sharply acute to subacuminate. 38. *V. mattogrossensis.*

Middle and outer scales subacute to obtuse or rounded. 39. *V. baccharoides.*

Middle and outer scales lanceolate, acuminate and prolonged, with the prominently carinate midvein, into a subulate tip.

Leaves silvery-whitened beneath with dense but short sericeous pubescence, narrowly oblong-lanceolate, acuminate, narrowed to the base. 40. *V. Bakerana.*

Leaves not densely and closely silvery beneath.

Pappus deep brown.

Leaves softly and closely sericeous beneath with long hairs; lateral veins prominent, conspicuously equidistant and parallel.

Heads 11-flowered; outermost involucral scales mostly appressed, lanceolate, sharply tipped; stem and branches velutinous; leaf pubescence brownish, densely sericeous or subtomentose. 41. *V. densipaniculata.*

Heads 18–21-flowered; outermost involucral scales loosely erect, acicular; stem and branches densely sericeous with erect hairs; leaf pubescence gray, comparatively loose. 42. *V. mapirensis.*

Leaves pubescent beneath with comparatively sparse irregular hairs; lateral veins not conspicuously equidistant and parallel.

Involucre densely villous or subtomentose; stem closely and densely velutinous with brown hairs. 43. *V. sordidopapposa.*

Involucre thinly villous to nearly glabrous; stem villous. 44. *V. tristis.*

Pappus white or pale straw-colored.

Leaves of a lanceolate type, broadest below the middle, pale green or yellowish-green, densely or softly sericeous beneath; lateral veins prominently parallel.

Leaves rugose above and minutely scabrellate, the largest up to 15 cm. long; lateral veins scarcely elevated above the lower surface; stem velutinous with greenish-brown hairs; involucre brown, the inner scales mostly very thin and frequently somewhat spreading. 45. *V. pseudomollis.*

Leaves flat above and scabrously puberulent, the largest exceeding 20 cm. in length; lateral veins 20–25 pairs, stout, heavy, conspicuously elevated above the lower surface; involucre purplish, the inner scales sharply acuminate and strictly erect. 46. *V. costata.*

Leaves of an elliptic type, broadest near the middle, dark green, resinous beneath, pubescent or barely sericeous on the lower surface and prominently so on the veins; lateral veins not conspicuously parallel. 47. *V. cordiaefolia.*

29. *V. echitifolia* Mart., DC. Prodr. **5**: 60. 1836.
Cacalia Hieronymi Kuntze, Rev. Gen. 3^2: 138. 1898.
Velasco, Kuntze.

30. *V. herbacea* (Vell.) Rusby, Mem. Torrey Bot. Club **4**: 209. 1895.
Chrysocoma herbacea Vell., Fl. Flum. 330. 1825.
Vernonia obovata Less., Linnaea **4**: 279. 1829.
V. paucifolia Rusby, Mem. Torrey Bot. Club **3**: 50. 1893.
Yungas, Bang *247;* North Yungas, Buchtien *4415;* Coroico, North Yungas, Buchtien *3929;* Sorata, Larecaja, Mandon *235;* Reyes, Rusby *1316*, *1715*, *1726*.

31. *V. santacruzensis* Hieron., Engler's Jahrb. **22**: 699. 1897.
Sierra de Santa Cruz, Kuntze (type or isotype).

32. *V. ferruginea* Less., Linnaea **4**: 271. 1829.
Coroico, Yungas, Bang *2420*.

33. *V. crassifolia* Rusby, Bull. N. Y. Bot. Gard. **8**: 124. 1912.
Apolo, Williams *1513* (type).

34. *V. membranacea* Gardn., Hook. Lond. Jour. Bot. **5**: 217. 1846.
V. ruficoma Schlecht., Mart. Fl. Bras. 6^2: 105. 1873.
V. rufo-papposa latifolia Hieron., Engler's Jahrb. **22**: 700. 1897.
V. squamipes Rusby, Bull. N. Y. Bot. Gard. **8**: 124. 1912.
Apolo, Williams *1410*, *34;* Velasco, Kuntze (type or isotype of *V. rufo-papposa latifolia*); between Guanai and Tipuani, Bang *1420;* Tumapasa, Williams *522* (type of *V. squamipes*).
V. squamipes seems to be merely an abnormal form of this species, with the heads raised on scaly pedicels, as was noted by Ekman.

35. *V. brasiliana* (L.) Druce, Bot. Exch. Club British Isl. **3**: 426. 1914.
Baccharis brasiliana L., Sp. Pl. ed. 2. 1205. 1763.
Vernonia scabra Pers., Syn. **2**: 404. 1807.
Reis, Rusby *1589;* Canamina, Rusby *45*, *313*.

36. *V. myriocephala* DC., Prodr. **5**: 40. 1836.
Cochabamba, Bang *1207;* Antahuacana, Buchtien *2300;* near Mapiri, Buchtien *1530*, *1531;* Espia, Rusby and White *304;* Bopi River, Rusby *598*, *725;* without locality, Mulford Expedition *2149*, *718*, *701*.

37. *V. Lehmanni* Hieron., Engler's Jahrb. **19**: 44. 1894.
Sierra de Santa Cruz, Kuntze.

38. *V. mattogrossensis* Hieron., Engler's Jahrb. **22**: 696. 1897.
W. Velasco, Kuntze.

39. *V. baccharoides* HBK., Nov. Gen. & Sp. **4**: 40. 1818.
V. Bangii Rusby, Mem. Torrey Bot. Club **6**: 52. 1896.
Charopampa, Williams *791;* San Carlos, near Mapiri, Buchtien *1535*, *5734;* between Mapiri and Tipuani, Bang *1483* (type of *V. Bangii*); Espia, Rusby and White *110*, White *606;* Canamina, Rusby *310*, White *253*.

40. *V. Bakerana* Britton, Bull. Torrey Bot. Club **18**: 331. 1891.

Yungas, Rusby *2147* (type); Santa Cruz, Williams *1450;* Apolo, Williams *2452;* Coripati, Bang *2189.*

41. *V. densipaniculata* Rusby, Bull. N. Y. Bot. Gard. **8**: 126. 1912.

Cargadira, Williams *1534* (type).

Close to, if not identical with, *V. velutina* Hieron., authentic material of which has not been seen.

42. **V. mapirensis** n. sp.

Stems shrubby, 4 m. high, densely sericeous with erect, light-brown hairs; leaves thin, deep green, oblong-elliptic, the largest 15 cm. long by 5 cm. wide, acute or barely acuminate, entire, cuneate at base, on petioles 5–8 mm. long, glabrous and somewhat rugose above, paler green and shining below with dense sericeous pubescence, which is especially prominent on the conspicuous, parallel, curved-ascending lateral veins; inflorescence terminal, freely branched, many-headed, bearing reduced leaves at the bases of the principal branches; heads 18–21-flowered, sessile, 8–20 mm. apart; involucre broadly campanulate when pressed, 6–7 mm. high; involucral scales loosely erect, or the outermost somewhat spreading, the inner narrowly lanceolate and acuminate, the outer acicular with a prominent carinate midvein, hirsute, especially above; corollas violet; achenes densely hirsute with erect hairs, about 2.5 mm. long; pappus dull brown, the bristles 5 mm. long.

Vicinity of Mapiri, Buchtien *2462, 1533* (type, in the herbarium of the New York Botanical Garden).

43. *V. sordidopapposa* Hieron., Engler's Jahrb. **22**: 697. 1897.

Without precise locality, Kuntze (type or isotype).

44. *V. tristis* Hieron., Engler's Jahrb. **22**: 683. 1897.

Valley of the Rio Junta, Kuntze (type or isotype).

45. **V. pseudomollis** n. sp.

Stems closely and softly velutinous with yellowish-brown hairs; leaves firm, yellowish-green, the blades lanceolate, 10–15 cm. long by 2.5–4.5 cm. wide, acuminate, entire, abruptly narrowed to an obtuse or somewhat rounded base, on petioles 8–15 mm. long, above rugose and softly pubescent when young, becoming in age scabrellate with the persistent hair-bases, beneath densely and softly sericeous with grayish-white hairs; lateral veins 10–15 pairs, curved-ascending and prominently parallel, impressed above, barely elevated above the pubescence beneath; inflorescence terminal, freely branched, bearing a few reduced leaves at the nodes; heads sessile, about 21-flowered, 5–15 mm. apart; involucre broadly campanulate, 5–6 mm. high, varying from almost glabrous to densely pubescent; outer scales subulate, the middle long-acuminate with a prominent carinate midvein, the inner sharply acute or acuminate, flat, thin, and frequently spreading; achenes 1.5 mm. long, sericeous-hirsute; pappus white or pale straw-colored, the bristles 4.5–5 mm. long, the paleae oblong-linear, 0.7 mm. long.

Yungas, alt. 6000 ft., Rusby *1658* (type, in the herbarium of Columbia University); Apolo, Williams *1432.*

46. *V. costata* Rusby, Mem. Torrey Bot. Club **6**: 53. 1896.
Between Mapiri and Tipuani, Bang *1472* (type).

47. *V. cordiaefolia* HBK., Nov. Gen. & Sp. **4**: 38. 1818.
V. patuliflora Rusby, Bull. N. Y. Bot. Gard. **4**: 376. 1907.

This common species presents a considerable range of variation, but is nevertheless readily recognizable and constant to the characters set forth in the key.

Coroico, Yungas, Bang *2396* (type of *V. patuliflora*), Buchtien *3931*, *3930;* Antahuacana, Buchtien *2256;* San Carlos, near Mapiri, Buchtien *1532*, *1534*, *2462*.

Subsection 3*f*

Leaves auriculate-clasping at the base.
- Involucre 5 mm. high, its scales few, loosely imbricate in few series, narrowly lanceolate, sharply acute; leaves narrowly elliptic, toothed above the middle to the short-acuminate tip, abruptly constricted below the middle, nearly glabrous beneath. 48. *V. prenanthoides.*
- Involucre 13 mm. high, its scales numerous, closely imbricate in several series, oblong, the inner obtuse or minutely apiculate; leaves narrowly oblong, 15–20 cm. long, sharply toothed, densely pubescent beneath. 49. *V. auriculata.*

Leaves not auriculate-clasping.
- Involucral scales all acute, or the inner lance-subulate, purple; leaves ovate, strictly entire, on petioles 2–3 mm. long. 50. *V. laurifolia.*
- Inner involucral scales blunt, the outer acute to cuspidate, green, or with purple tips; leaves not ovate.
  - Leaves narrowly oblong; involucral scales in relatively few series. 51. *V. canaminina.*
  - Leaves broadly oblong to elliptic; involucral scales in numerous series. 52. *V. fulta.*

48. **V. prenanthoides** n. sp.

Stems shrubby, 2 m. high, the younger portions glabrous, prominently angled; leaves closely sessile, auriculate-clasping, elliptic, abruptly short-acuminate, sharply serrate above the middle, entire and conspicuously constricted below the middle, glabrous above, minutely puberulent with short conic hairs beneath, pinnately veined; inflorescence large, paniculate, freely branched, the cymes short, with few heads; heads about 18-flowered, sessile or on peduncles up to 3 cm. long; involucre broadly obconic, 6 mm. high, its scales irregular in length, loosely imbricate below, erect, few in number, not fully concealing the achenes, straw-colored or dull brown, lanceolate, glabrous, the outer acuminate, the inner sharply acute, achenes prominently ribbed, short-hirsute; pappus straw-colored, the bristles fragile, 5 mm. long, the paleae 0.5 mm. long.

Rurrenabaque, alt. 1,000 ft., Rusby *756* (type, in the herbarium of the New York Botanical Garden), Rusby *757*.

The leaves are closely similar in outline and serration to those of *Aster*

prenanthoides; the largest in the two sheets at hand are 11 by 4 cm. Corollas are not present, but were noted by the collector as blue or white in color.

49. *V. auriculata* Griseb., Goett. Abhandl. **24**: 163. 1879.

Tarija, Lorentz *874* (isotype).

50. *V. laurifolia* DC., Prodr. **5**: 30. 1836.

Cargadira, Williams *1531;* Yungas, Bang *617*, Rusby *1617;* Mapiri, Bang *1534*.

51. **V. canaminina** n. sp.

Herb 6–9 dm. high, with purple flowers; stems strongly angled, glabrous, branching only in the inflorescence; leaves linear-oblong, the upper about 6 cm. long by 8 mm. wide, sharply but remotely dentate, closely sessile, scabrellate above, closely and finely tomentulose beneath; inflorescence large, loose and open; heads numerous, sessile or peduncled, about 26-flowered; involucre 8 mm. high, broadly obconic to subhemispheric, the few scales loosely and irregularly imbricate, glabrous, green or brownish below, purple at the tip, with a green or scarious margin, the outer ovate-lanceolate, acute or subacute, the inner narrowly oblong, obtuse or rounded; corolla 8 mm. long, the linear lobes glabrous, 2.5 mm. long; achenes 4 mm. long, prominently ribbed, pubescent; pappus pale straw-colored, the numerous bristles 7 mm. long, the paleae linear, erect, 0.6 mm. long.

Canamina, alt. 4,500 ft., White *752* (type, in the herbarium of the New York Botanical Garden).

Differing from *V. fulta*, with which it appears related, in its narrower and sessile leaves, its erect habit, tomentulose leaves, broader and more open inflorescence, smaller heads, shorter involucre, narrower and blunter involucral scales, and shorter pappus. The type specimen bears only two leaves, so that the shape and dimensions of the lower ones are unknown.

52. *V. fulta* Griseb., Goett. Abhandl. **24**: 164. 1879.

V. senecionaefolia Britton, Bull. Torrey Bot. Club **18**: 331. 1891.

V. trixioides Rusby, Mem. Torrey Bot. Club **6**: 54. 1896.

Mapiri, Williams *782*, Bang *1484* (type of *V. trixioides*), Buchtien *1523;* Guanai Rio, Williams *1593;* Machichoirisa, Williams *1607;* Canamina, Rusby *312;* Yungas, Rusby *1730* (type of *V. senecionaefolia*); Suri, White *226;* Bopi River, Rusby *599;* without definite locality, Kuntze, Mulford Biological Exploration *2148*.

In addition to the preceding, *V. mollis* HBK. was reported from Bolivia by Schultz-Bipontinus, who also published the *nomina nuda V. centauroides* and *V. quindecimflora*. No material has been seen which could be referred to *V. brachylepis* Griseb. or to *V. centauropsidea* Hieron.

New York Botanical Garden

STUDIES OF PHYSICAL AND MORPHOLOGICAL CHANGES IN BARTLETT PEARS[1]

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INTRODUCTION

During the comparatively short period of development and maturity, the pear fruit passes through a series of profound morphological, physical, and chemical changes. While the gross features of most of these alterations are more or less observable, the more detailed changes, such as may have taken place during a brief interval of time, are rather difficult to measure or even to approximate. Yet in all harvesting, transportation, and storage operations with most of the commercial varieties of pears, these changes assume a profound importance, for they have a direct bearing on both the eating and the keeping quality of the fruit. This is of particular significance in view of the fact that the pear is a highly perishable product. Hence of late, because of the rapid expansion of our marketing operations, particularly with fruit from the Pacific Coast, the subject is receiving an ever-increasing emphasis. There is a growing demand for more extensive and more detailed information in this field as a logical addition to some of the groundwork already laid by a number of more or less correlated investigations.

REVIEW OF LITERATURE

As a result of the studies of Kulisch (9), Ewert (2), Kelhofer (6), Ritter (15), Rivière and Bailhache (16), and other European investigators, there has accumulated considerable information on the chemical changes in the pear as affected both by seasonal differences and by artificial alterations of a number of environmental factors. In this country, Dunbar and Bigelow (1), Thompson and Whittier (17), and Magness (11) have made valuable contributions on the subject. All of these investigations, however, have been based largely on changes of the cell contents and only indirectly on those of the structural part of the tissues. This is but natural if we remember that our present knowledge of the chemistry of the principal substances of the cell walls is very imperfect. So, too, the culinary properties of the pear fruit being determined primarily by the amount of sugars, acids, and tannin present, these substances have naturally received first consideration. Though most of our present chemical data have been secured from samples taken at considerable intervals of time, still they may

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be used as fairly good indices of the gross seasonal changes of the fruit during the time both of development and of maturity. It is to be questioned, however, whether it would be at all possible to show by means of chemical analyses such small differences in maturity as take place in but a few, say three or four, days.

In contrast to our knowledge of the chemistry of the pear, we know far less of the important morphological and physical changes that are coincident with the development of this fruit. The fundamental work of Kraus, McAlpine, Malfatti, Zschokke, and others is, however, of particular significance in this respect. They have given us a good conception of the gross structure of the pear fruit. Hence their work warrants a detailed review.

Though not considering the pear directly, the analytical researches of Kraus (7) on the morphology of pomaceous fruits convey thorough and definite information on the character and development of the various tissues—vascular, parenchymatous, dermal—forming a pome fruit. Briefly, it is to be regarded as consisting of several drupe-like fruits, borne within, and connected with, a fleshy torus. The three regions—carpellary, pith, and cortical—are clearly distinguishable both as to their ontogenetic development and their morphology. Outside of the seed, development of the structures consists mainly in the expansion of cells already formed, alterations in their chemical content, and the storing of food. It is hinted, however, that further cell division may possibly take place in some such manner as suggested by Farmer (3). The vascular system permeates all parts of the fruit except the pith region, the cortex being particularly well supplied. The ten primary toral bundles divide and anastomose, forming a complete fibral network under the epidermis.

The fibro-vascular system of the pear (pome) as it appears in two varieties, Harrington's Victoria and Achan, has been pictured by McAlpine (13). Excepting the pith region, it forms a complete and elaborate network throughout the fleshy part of the fruit.

Malfatti (12) describes more in detail the anatomy of the pear, particularly that of the epidermal region. As a result of growth, epidermal cells divide into "daughter" cells. Thinness of the walls indicates which are the newest cells. A thick cuticle covers the epidermis. It becomes cracked and torn at points where lenticels are formed. The latter are underlain with cork. So, too, in case of russeting, epidermal cells anywhere may be replaced by cork cells. Below the epidermis is found a subepidermis consisting of 3–4 layers of tangentially stretched plate-like parenchymatous cells. Ordinary isodiametric cells of the cortex adjoin the subepidermis. In addition to protoplasm, they contain small granules of starch, distributed either singly or in groups, and sugar in solution, while subepidermal cells contain beside sugar also chlorophyll, tannin, and chromoplasts. Stone cells, either singly or more frequently in groups, are found imbedded in

the cortex. They are roundish-polygonal, very thick, and are layered and dotted with many pore canals. Radially arranged parenchymatous cells adjoin groups of stone cells.

The structural features of the epidermis are described in further detail by Zschokke (19). Increase of the surface of the fruit is accomplished partly by subdivision and partly by expansion of the epidermal cells. Tangential walls of the epidermis become thickened early in the development of the pear. Great differences in amount of cuticle were observed between fruit grown in the sun and that grown in the shade. The "bloom" consists of particles of wax. Stomata are present in all young fruit. As the fruit grows, they disappear or are torn and become lenticels. Morphologically, the subepidermis differs considerably from that of the cortex. The cells are smaller, more tabular, thick-walled, and contain tannin and pigment bodies. Color of fruit depends on the contents of these cells. Within the subepidermis are found scattered stone or grit cells. They are plated on the outside and pointed toward the core. This layer of stone cells is present in all pears. In some it forms almost a "stony shell" all over the fruit.

It is to be noted that none of the above-named investigators take cognizance of such morphological or physical alterations in the pear as may be exhibited comparatively late in the development of the fruit and may therefore be coincident with the time of harvesting and marketing of the crop. No correlation is noted between the morphological features and the condition of maturity of the pear.

A Physical Test for Maturity

In view of the existing situation, the Oregon Agricultural Experiment Station has for the past four seasons endeavored to find a simple but reliable test for maturity of pears. As the work progressed, it was soon evident that such a test, in order to be applicable, must be based largely, if not entirely, upon the physical properties of cells rather than upon their chemical contents. Of the various new testing methods under consideration, a simple one, since then known as the "pressure test," has given strikingly satisfactory results. Work extending through four seasons has shown that it is by far the most practical means of measuring the changes in maturity of pears (10, 14).

This new test is based upon the fact that during the growth and ripening of the pear there is a gradual and consistent alteration in physical resistance to pressure or wounding of the epidermal and cortical regions of the fruit. On the average this amounts to close to one half pound every 24 hours. It points either to rapid changes in size or to some other modifications of the structural parts of the cells. A special apparatus was constructed for the purpose of measuring and expressing in convenient units this change.[2]

[2] For a description of the apparatus and of its use, see Oregon Agricultural Experiment Station Bulletin 186, pp. 7–10.

During the past four shipping seasons several thousand tests have been made by means of this instrument on all leading commercial varieties of pears in Oregon, but particularly on the Bartlett.

TABLE 1. *Summary of Pressure Tests, Bartlett Pears, Oregon, 1918–1921*

Date	Rogue River Valley		Willamette Valley	
	1918 Ave. of 4 orchards, Lbs.	1919 Ave. of 5 orchards, Lbs.	1920 Ave. of 4 orchards, Lbs.	1921 Ave. of 3 orchards, Lbs.
August 1	34.5			
August 2	32.4	35.7		
August 4		35.1		
August 5–6	31.2	35.1		
August 8–9	30.0	34.0		
August 11		32.1		41.0
August 12	29.2			
August 13		31.7		
August 15	27.8	31.7		
August 16				36.7
August 18		30.5		
August 19–20	25.4		40.8	36.2
August 22	24.4	29.4		
August 23			38.5	33.8
August 25		27.8		
August 26	24.3			32.2
August 27			37.5	
August 29		26.5		
August 30			36.7	31.0
September 2			34.5	30.8
September 6				29.3
September 7			33.2	
September 11			31.6	
September 14			27.2	

Table 1 shows in a condensed numerical form the results of several thousand tests on Bartletts. It is evident that a strict correlation exists between the stage of maturity of a Bartlett pear and the resistance to physical pressure of the outer regions of the fruit. The data for the respective years show a really remarkable consistency in this respect (text fig. 1). It should be particularly noted that the data were collected at extremely short (2- to 4-day) intervals. These results have been put to practical test in a number of commercial orchards. The "pressure apparatus" is now in use in several pear-growing sections in the Pacific northwest. It permits the individual grower, or the growers of a certain district, to measure conveniently and to express in concrete units the exact stage of ripeness of the fruit at the time of harvesting or shipping. One may thus be guided in all harvesting operations and helped to avoid misunderstanding and unnecessary economic loss.

In connection with the above-described investigations, the question

naturally arose as to what may be the underlying causes of the gradual change of tissues of the epidermis and the cortex to resistance to pressure of the character above noted. One is led to assume that the factors involved

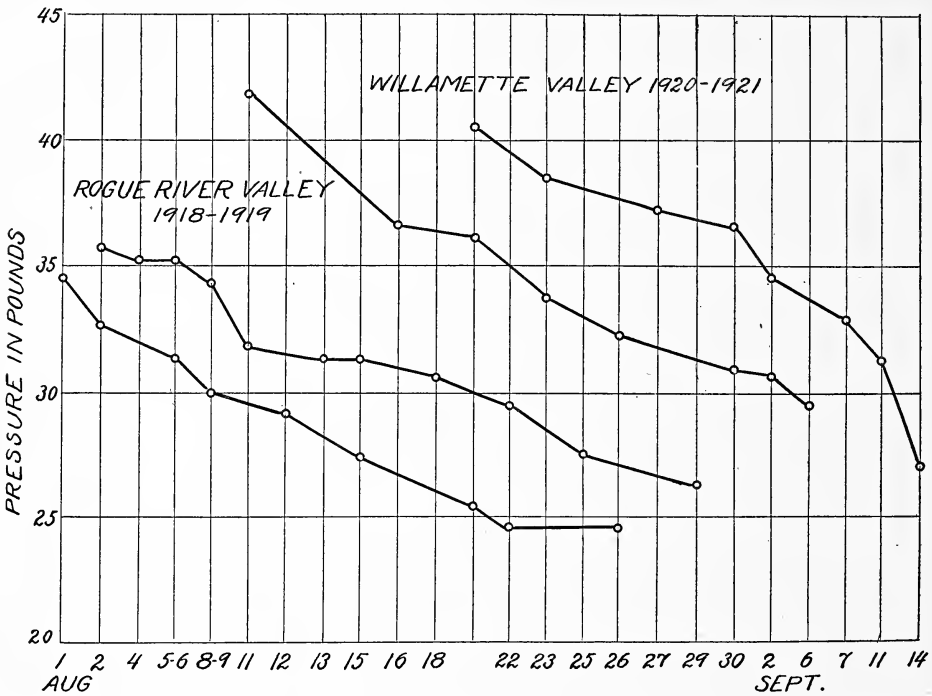

FIG. 1. Graphs showing records of pressure tests on Bartlett pears and length of shipping seasons. Rogue River Valley, Oregon, 1918–1919, and Willamette Valley, Oregon, 1920–1921.

are primarily physical, but in accordance with their physical alterations cells undergo considerable chemical changes. No chemical investigations, however, were attempted at this time. The following is a report on studies of morphological and histological modifications of the Bartlett pear as revealed during the comparatively brief period of harvesting and shipping of the fruit.

MORPHOLOGICAL AND HISTOLOGICAL INVESTIGATIONS

Material and Methods

A concise account may be given here of the nature and extent of injury caused by the physical test, *i.e.*, by the tip of the plunger of the pressure apparatus. The tissues affected form a conical area, with the apex toward the axis of the fruit (Pl. XXIV, figs. 4, 5). The epidermis, subepidermis, and a considerable number of immediately underlying cells of the cortex are not ruptured, excepting in extremely ripe pears, but are torn in a circle, forming a plug which crushes against the cells of the cortex. A decided line of

rupture is thus caused from the epidermis down into the flesh around the outer margin of the area of pressure. As the fruit increases in ripeness, much more crushing of cells of the cortex takes place. The cells adjoining the line of cleavage do not appear to have been pressed out of position. Many of them perforce are torn, or else they may be separated from one another along the middle lamellae. It is to be noted that in no fruit used in connection with this study did the area of pressure extend farther than the cortical region. Consequently the present histological study was confined to the cortex and epidermis.

Bartlett pears, obtained from four typical orchards and employed for the pressure tests, were used as material. Radial sections (plugs) from representative specimens of each orchard were cut at the point of the greatest diameter of the fruit at the time of taking of pressure records (fig. 5). They were preserved in 70% alcohol. All material for microscopic observation was imbedded in paraffin. Radial transverse sections were cut 10 and 25 microns thick, stained in Delafield's haematoxylin or safranin, or in both for contrast, and preserved in Canada balsam. In all cases standard histological methods were followed.

Results

Upon examination of a large number of sections, it was soon evident that in order to show the greatest possible differences in all measurements only material collected comparatively early and comparatively late in the season should be taken into account. Hence, "early" refers here to sections obtained from specimens preserved on August 20, and "late" to those preserved on September 17. These dates indicate the greatest extremes of the harvesting season for the Bartlett pear in the Willamette Valley, Oregon, in 1920.

Cuticle. Early in the season the cuticle is quite uniformly distributed over the epidermis, filling the spaces between the unevenly arranged cells. Opposite a comparatively wide space, formed by grouping of cells, the cuticle is slightly depressed or somewhat torn. As the season progresses, narrow cracks appear. The outer surface now becomes more uneven and "netted." This condition increases as the fruit enlarges, some specimens showing a decidedly close-netted checking during or towards the end of the ripening time. Under certain circumstances these may turn into open cracks with slightly raised ridges. There is a general decrease in thickness of the cuticle.

TABLE 2. *Measurements of Thickness of Cuticle*

Date	Aug. 20	Sept. 17	Decrease	Ripe
No. of determinations	35	34		15
Thickness	5.8 μ	4.7 μ	19.0%	4.2 μ

Epidermis. Because of differences in position, size, and form of the cells, only the outermost layer of cells has been designated here as *epidermis.* Great irregularity in form and distribution is exhibited by the cells of this layer. Many of them are tangentially stretched, or become so in due course of time. A considerable number, however, are conic in form, with the apex protruding into the cuticle. Tangential and radial measurements taken early and late may be considered as a fair index of the general change in form and size of the epidermal cells. The outer tangential wall is usually somewhat thickened, and the thickening may extend also to the fore part of the radial wall. No seasoned change in this respect, however, could be detected.

TABLE 3. *Measurement of Cells of Epidermis*

	Ave. Diam., Tangential	Ave. Diam., Radial	Approx. Area Long. Sect.	Increase
No. of determinations....	42	42		
August 20.....	13.5 μ	12.5 μ	170 sq. μ	
September 17.....	15.5 μ	12.5 μ	194 sq. μ	14.1%

Two or more cells, often called "daughter" or "window" cells, may be grouped together to form a "mother" cell, as shown by McAlpine (13) and other investigators, thus making a comparatively large open space between adjacent groups. Early in the growth of the fruit the "window cells" appear to be more irregular in outline and closer together. The average number of "window cells" per "mother cell" was found to have increased during the season as shown in table 4. Material collected on August 20 showed

TABLE 4. *Average Number of "Window Cells" per "Mother Cell"*

	Date	
	Aug. 20	Sept. 17
No. of determinations.....	53	53
Number.....	2.92	4.00

usually two "window cells" in each group, though some groups contained four, and a few one, three, or five cells. On September 17 the average number of "daughter cells" had increased to four, with a few groups containing two, six, and eight. Uneven numbers of cells—three, five, etc.—were now only rarely encountered. This numerical distribution may be considered as a fair proof of the usual subdivision of the original cells during the time of enlargement of the fruit. Quite often single undivided cells were also present.

Subepidermis: First layer. Though the tissue to a depth of five cells or more immediately below the epidermis may be designated as *subepidermis* (fig. 6), the first layer was considered separately. Cells of this layer differ

quite conspicuously from those underlying them, being much smaller and more flattened. Stretched tangentially and plate-like in form, they are more evenly arranged and more closely united with each other than are those of the epidermis. There appears to be no thickening of the outer walls. Subepidermal cells of the first layer show a proportionally faster seasonal increase in tangential than in radial diameter. The resultant enlargement in size, as measured in one plane, is close to 40% (table 5). This perhaps is a good indication that subdivision of cells is much less frequent here than in the epidermis.

TABLE 5. *Measurement of Cells of Subepidermis (second layer below epidermis)*

	Ave. Diam., Tangential	Ave. Diam., Radial	Approx. Area Long. Sect.	Increase
No. of determinations....	80	80		
August 20.....	18.2 μ	12.2 μ	224 sq. μ	
September 17.....	22.2 μ	14.0 μ	312 sq. μ	39.3%

Second to fifteenth layers. As a rule, cells of the subepidermal region, as far as the fifteenth layer, are considerably smaller and more compact than those of the cortex proper (fig. 6). Observation showed, however, rather gradual but quite conspicuous increments in size from the periphery inward. Most probably the enlargement is at the same rate in all diameters. From measurements in 1920 it appears, however, that a relatively greater increase had taken place in the radial diameter, the cells having rounded out and become more nearly isodiametric. No difference in thickness of walls on the peripheral side of the cells could be noted.

TABLE 6. *Measurements of Cells of Subepidermis (3d to 5th layer below epidermis)*

	Ave. Diam., Tangential	Ave. Diam., Radial	Approx. Area Long. Sect.	Increase
No. of determinations....	80	80		
August 20.....	26.5 μ	16.8 μ	445 sq. μ	
September 17.....	33.5 μ	22.0 μ	737 sq. μ	65.6%

Within the subepidermis are scattered groups of a few to a dozen or more sclerenchymatous or stone cells (fig. 7). Occasionally single isolated cells of this type are found imbedded in this region. Although the sclerenchymatous cells of the subepidermis are conspicuous enough in the Bartlett, Zschokke (19) found them to be so numerous in some late varieties of pears as to form a shell as it were around the fruit. No seasonal change in distribution of stone cells could be detected, though naturally they become somewhat more

widely separated due to the expansion of parenchymatous cells surrounding them.

Lenticels. As a result of the expansion of the epidermis, the stomatal openings are pulled apart, resulting in the formation of distinct pores or lenticels. During the fore part of the season (August 20) they are comparatively small in size and conspicuously deep (fig. 8). Cork cells are then distributed largely around the edges of lenticels. As the season progresses, the lenticels gradually become larger and shallower and more cork cells are laid down at the bottom. The fruit developing still further, lenticels eventually become flat, shallow openings surrounded and underlain with cork tissues. Extreme expansion and mass formation of corky tissues may eventually disfigure a lenticel, leaving only a patch of corky tissues with a marginal scar of the epidermal cells surrounding it (fig. 9).

The seasonal changes in relative distribution of lenticels may be of interest. A large number of close determinations showed that the average number of lenticels per square centimeter of surface area of the fruit had decreased between August 20 and September 14 from 44 to 32.

TABLE 7. *Number of Lenticels per Square Centimeter of Surface of Fruit*

	Date	
	August 21	September 14
No. of determinations	13	9
No. of lenticels	44	32

Cortex. The pressure test involves primarily the cortical cells. Hence, from the point of view of physical changes in the pear the cortical region is of far greater importance than any other heretofore considered. Moreover, the tissues of the cortex constitute the main edible part of the fruit. Naturally any alterations that may take place here will at once affect its keeping and eating qualities. The cells of this part of the pear are extremely thin-walled, more or less nearly isodiametric in form, and quite variable in size (fig. 10). While arranged rather compactly early in the season, they become separated at maturity of the fruit, giving rise to the large intercellular spaces. During the enlargement of the pear, cortical cells undergo a rapid increase in size.

TABLE 8. *Measurements of Cells of Cortex (approximately 3–6 mm. below epidermis)*

	Ave. Diam., Tangential	Ave. Diam., Radial	Approx. Area Long. Sect.	Increase
No. of determinations	80	80		
August 20	90.5 μ	130.8 μ	1188 sq. μ	
September 17	128.8 μ	178.8 μ	2301 sq. μ	93.7%

Groups of stone cells of considerable size are found scattered throughout

this region. They are surrounded by radially arranged, distinctly elongated paranchymatous cells (fig. 11). There is considerable variation in the number and distribution of these groups in the various specimens. As the cells of the cortex enlarge, the groups of stone cells in a given area become fewer.

The wound caused by the pressure test being of such a character as to tear the cells across their walls instead of separating them from one another along the middle lamellae, any change in the walls becomes of prime importance in these considerations. Measurements showed a general decrease of close to 15% in thickness of walls of the cortical cells during the harvesting season. It is certain, however, that, in addition to changes in thickness,

TABLE 9. *Measurements of Cell Walls of Cortex (approximately 3–6 mm. below epidermis)*

	Date		
	Aug. 2	Aug. 30	Decrease
No. of determinations	45	45	
Thickness	1.15 μ	0.98 μ	14.8%

other physical and chemical modifications of the cell walls take place at this time. Most probably changes in the pectic compounds are of prime importance in this respect. Since no tests were made, one can only conjecture what some of these differences may be.

Vascular bundles. The vascular system being made up of more lignified cells than any other tissues considered here (7, 13), one would expect it to undergo the least change during the time in question. No study was made of seasonal modifications in vascular bundles.

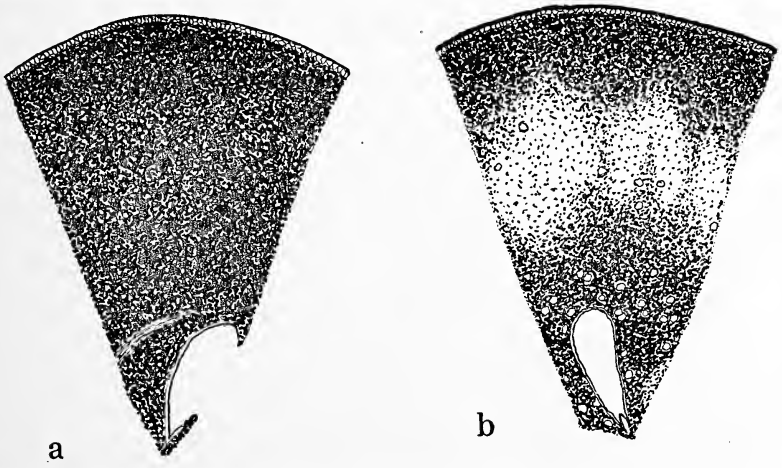

FIG. 2. Radial sections from the cortex stained with potassium iodid to show distribution of starch: *a*, 32 lbs. pressure; *b*, 34 lbs. pressure. Camera lucida drawings. × 25.

Cell contents: Starch. Of the various substances found within the cell, solids only are of importance in this study, since they only would have any bearing upon a physical test of this nature. Besides protoplasm and some minor substances, starch is the usual important solid in the cells of the cortical region of the pear. During the early part of the development of the fruit there is an abundance of starch in all tissues excepting the conducting vessels (text fig. 2, *a*). As the fruit matures starch disappears rapidly, the disappearance beginning with the region near the primary vascular bundles and thence continuing through the cortex to the periphery of the fruit. The final traces of starch are usually visible in the subepidermal region (text fig. 2, *b*). On August 20, starch grains, 6–11 microns in diameter and spherical or nearly so in form, were found either singly or in aggregate groups of several grains in almost all cells (text fig. 3, *a*).

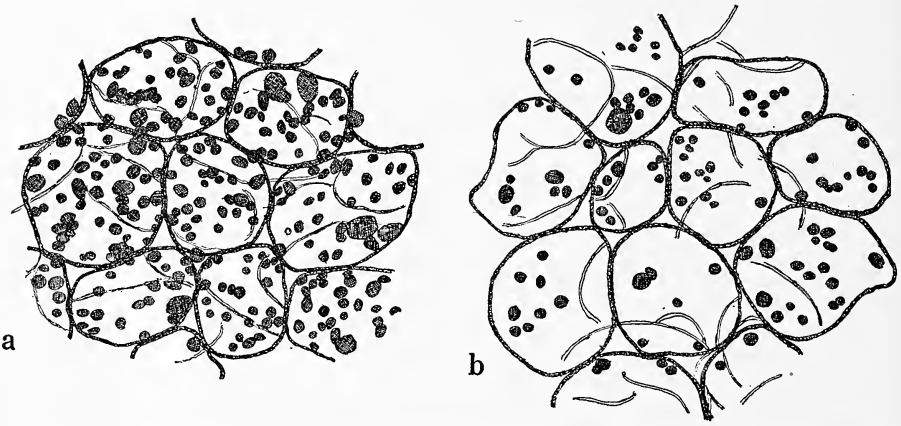

FIG. 3. Cells of Bartlett pears showing relative amounts and distribution of starch: *a*, Aug. 20; *b*, Sept. 17. Camera lucida drawings from one optical plane, more than one cell in thickness. × 200.

Actual counts and measurements of starch grains as found early and late in the season, 6–8 mm. below the epidermis, showed that the approximate calculated volume occupied by starch diminished from 5.49 percent to 1.17 percent (text fig. 3, *b*).

TABLE 10. *Number and Size of Starch Grains in Cells of Cortex (approximately 6–8 mm. below epidermis)*

	Ave. No. of Grains per Cell	Ave. Diam. of Grains	Percentage of Volume Occupied by Starch
No. of determinations.....	10	31	
August 20.....	54	8.7 μ	5.49
September 17.....	36	7.8 μ	1.17

It should be pointed out here that, when potassium iodid is used as an indicator of the amount of starch present, the sections will invariably stain solid blue as a result of the intensity of the color and the translucency of the cell walls, although only a small percentage of the volume (5 percent or less) is occupied by starch (text fig. 3, *a*).

Discussion of Results

From the foregoing data it may be concluded that a series of morphological and histological changes of the dermal and cortical regions of the pear may be largely responsible for the marked physical changes of these tissues in respect to resistance to pressure. Naturally the cells of the cortex are of the greatest importance here, since by far the predominating percentage of the tissues involved were those of the cortical region. While the average seasonal decrease in resistance to a certain physical pressure (14) amounted to approximately 30 percent, the cortical cells had increased in size during this period by 94 percent. At the same time, the walls of these cells showed a reduction of 14.8 percent in thickness. Consequently an assumption may be made of a probable correlation between the physical and the morphological changes in the cortex. One can only conjecture, however, as to the exact numerical relations here. So, too, it is possible that, as a result of a gradual hydrolysis of starch into sugars, a greater turgidity is attained by the cells of the cortex during the latter part of the season, resulting in a lowering of physical resistance. Such a mechanical explanation is offered by Hawkins and Sando (4) for the greater resistance to wounding of the epidermis of cherries and of various other small fruits. In the cases of these fruits, however, changes in turgidity, caused by lowering of temperature, are assumed to be due to probable differences in the coefficient of expansion of the cell walls and the cell contents. The point is very suggestive, since it has been shown by Lewis, Murneek, and Cate (10) that a marked increase in resistance to wounding of Bartlett pears was obtained when the fruit was kept for 24 hours at room temperature (summer). Naturally there was a considerable loss of moisture during this time, with a consequent profound effect on the turgidity of the cells.

Undoubtedly the chemical alterations of the various constituents of the cell walls should be considered here as an additional factor. It is a well known fact that the hemicelluloses, pentosans, and particularly pectic compounds, undergo radical changes at the time of maturity of the fruit. By careful chemical analyses, Magness (11), for instance, has shown that as the pear ripens on the tree there is a conspicuous decrease of alcohol-insoluble, acid-hydrolyzable substances other than starch. In many instances the decrease in these polysaccharides amounted to 50 percent of the total present, thus indicating that as the fruit develops much of this material, other than starch, is hydrolyzed. Magness points to the reduction of pectose and related material, which is thought to be largely responsible

for the thickening and cementing together of the cell walls, as a possible cause of the lowering of resistance to pressure of the pear.

There is a prevalent opinion that as the fruit ripens a gradual dissolving of the middle lamellae takes place (18, and others). Sections through the wounded areas of even comparatively ripe pears showed, however, that the cells had very rarely separated along the middle lamellae, but that the characteristic tearing was across the cell walls. This fact, of course, does not diminish the importance of the possible changes of pectic substances in the middle lamellae and the cell walls proper. Hornby (5), for instance, found an increased pectin content in those parts of the potato and other tubers that were most exposed to light. The writer's own experience indicates that the pigmented part of the pear, the side of the fruit most exposed to light, has a greater resistance to wounding than the green portion.

Of minor consequence here is the reduction of the starch content from 5.5 percent to approximately 1 percent of the total volume of the cell. This, too, may play a part in the relative resistance to physical changes of these cells. Again, because of expansion of the cortex and subepidermis, greater separation of the groups of stone cells may be effected, thus lowering to some extent the combined resistance to pressure of the tissues as a whole.

Apart from the cortex, the seasonal differences in size and distribution of the epidermal cells are of interest. Cells of the subepidermis show a relative increase of approximately 65 percent in size. Close to the outside this increase of course was much lower, amounting to 40 and 14 percent respectively for the first layer of subepidermis and epidermis. A decrease in thickness of the cuticle of 19 percent may also be noted. Undoubtedly all these factors contribute to the measurable physical changes of the tissues of this region. So, too, one may expect changes in the cell walls here quite similar to those suggested for the cortex proper.

In conclusion, it may be said that the lowering of resistance to physical pressure of the cortex and epidermis of the Bartlett pear may be due in a large measure to the morphological and histological changes of the tissues involved. In order of decreasing importance the factors under consideration could possibly be arranged as follows:

a. Cortex: Increase in size of cells and decrease in thickness of cell walls.

b. Subepidermis: Increase in size of cells and possible decrease in thickness of cell walls.

c. Epidermis: Increase in size of cells and decrease in thickness of cuticle.

d. Decrease in amount of solids (starch) in the cells.

e. A wider separation of the groups of stone cells in both subepidermis and cortex.

Summary

1. Changes in maturity of the pear fruit have been commonly expressed in terms of chemical differences of the cell contents.

2. A physical test has been perfected which shows that alterations in the structural part of the fruit are evidently more uniformly consistent than the chemical changes.

3. The results of several thousand tests with Bartlett pears showed a close correlation between physical resistance to a definite pressure or wounding of the epidermis and cortex, and the maturity of the fruit. When measured and expressed in convenient units, the average seasonal decrement in this respect was found to be approximately 10 lbs., or close to $\frac{1}{2}$ lb. for every 24 hours.

4. Though chemical modifications of the cell walls may be responsible to a large extent for these differences, the following seasonal morphological and histological changes were observed in the Bartlett pear:

a. There was an average increase of 93.7 percent in size and an average decrease of 14.8 percent in thickness of walls of cells of the cortex.

b. Cells of the lower portion of the subepidermis increased in size (area of a longitudinal section) by 65.6 percent, while those of the first layer beneath the epidermis increased by 39.3 percent.

c. Though comparatively slight, still there was a seasonal enlargement in size of epidermal cells amounting to close to 14 percent. The cuticle covering these cells decreased in thickness at the same time by 19 percent.

d. The average increase in number of "window cells" per "mother cell" was from 2.92 to 4.00.

e. Lenticels decreased during this period from 44 to 32 per square centimeter of surface area of the pear. A conspicuous alteration in form and structure of lenticels was noted.

f. The volume occupied by starch in an average cortex cell decreased from 5.49 percent to 1.17 percent.

5. A partial explanation of the significance of these morphological changes and their possible correlation with the physical resistance to pressure of the tissues involved is offered.

The writer wishes to express his sincere appreciation to Prof. E. M. Harvey for assistance with the histological work and for many helpful suggestions.

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DESCRIPTION OF PLATE XXIV

FIG. 4. Bartlett pear, showing position of pressure punctures. × 2/3.

FIG. 5. Section of Bartlett pear. Note character and depth of pressure punctures and section (plug) used for histological work. Natural size.

FIG. 6. The rather thick subepidermis of a Bartlett pear. × 30.

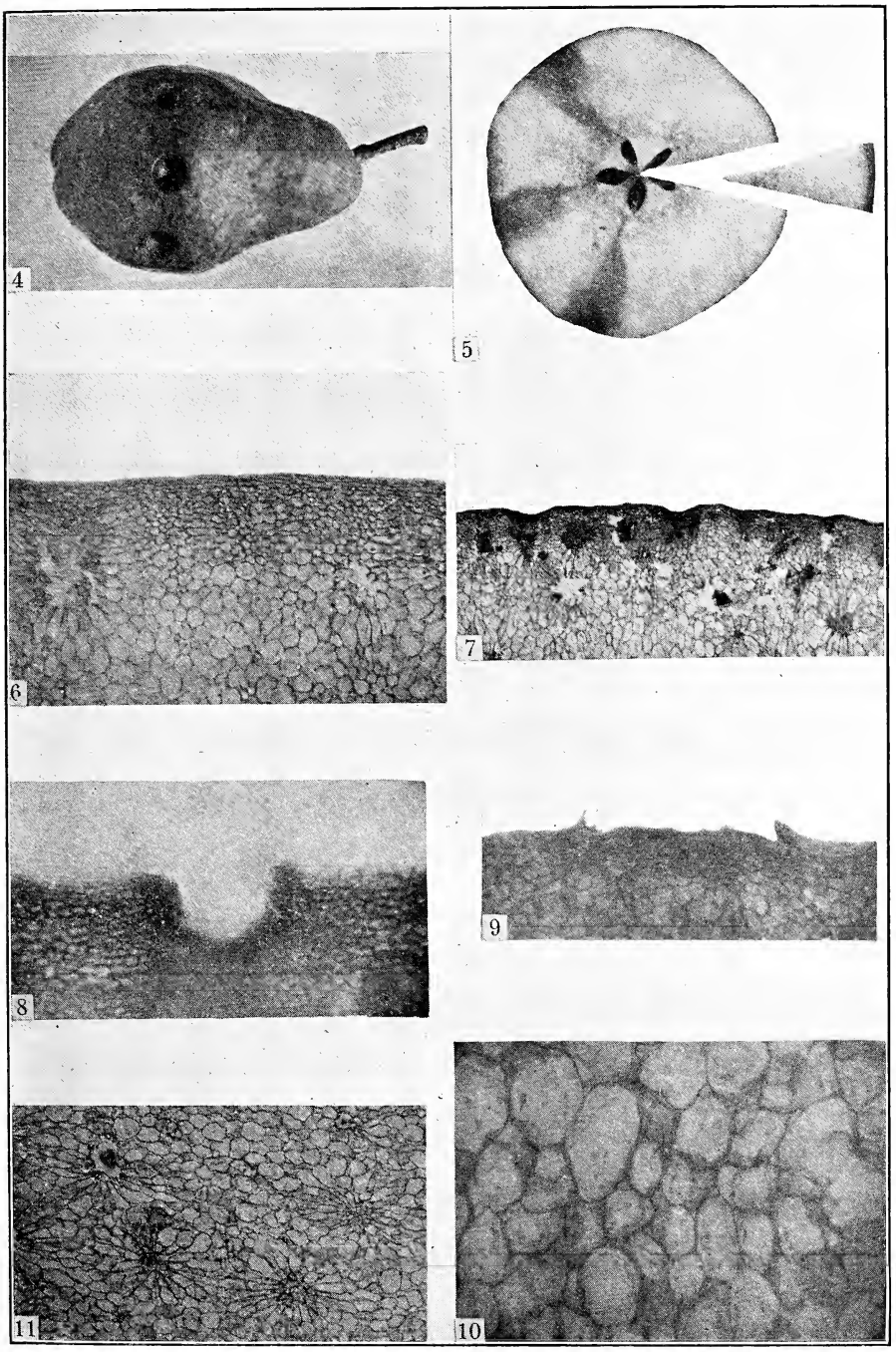
FIG. 7. Scattered groups of stone cells within the subepidermis. × 15.

FIG. 8. A typical lenticel early in the season (August 20). × 90.

FIG. 9. A typical lenticel late in the season (September 17). × 100.

FIG. 10. Cells of the cortex. × 125.

FIG. 11. Groups of stone cells in the cortex. × 25.



MURNEEK: CHANGES IN BARTLETT PEARS

THE FORMATION AND DEGENERATION OF GERM CELLS IN THE POTATO

W. J. Young

(Received for publication October 9, 1922)

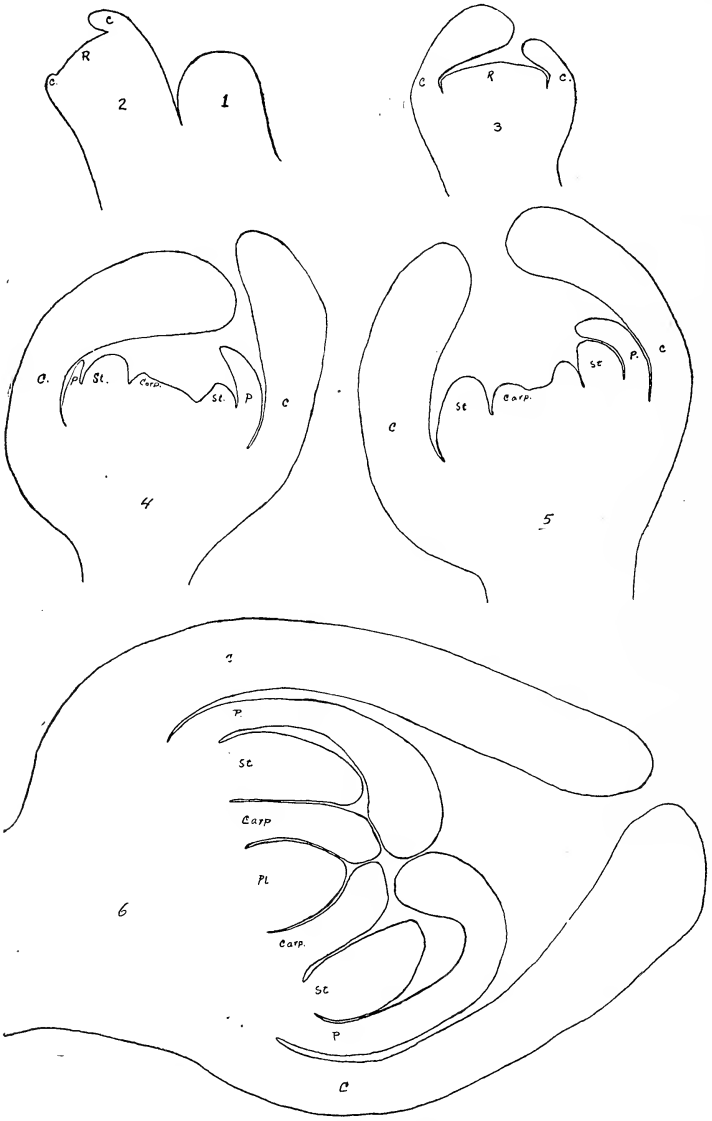
No information seems to be available on the cytological characters and germ-cell formation of the potato. Investigators who have undertaken preliminary work of this nature have found the subject unfavorable for study and have abandoned the project. The unfavorable characters are the small size of the cells and other structures, as a consequence of which it is difficult to make out the details, and the degenerative changes which occur in the germ cells and in associated structures as a result of congenital causes or of unfavorable conditions of environment.

The present study was carried out in connection with the Irish-potato-breeding project at the South Carolina Agricultural Experiment Station and is presented as a contribution from the Department of Horticulture of that institution. This paper is intended as a supplement to the material presented in Bulletin 210 of the Station, entitled, "Some Phases of Breeding Work and Seed Production of Irish Potatoes." The primary object of the work is to follow the degenerative changes in the germ cells on account of the bearing which such changes might have upon the failure of most potato varieties to set seed. As a matter of record and for purposes of comparison, the normal development of the floral structures and of the germ cells is described and figured in so far as these structures could be made out and as the available material permitted, although they conform quite closely to typical cases.

Development of the Flower

The flowers arise as small rounded masses of cells (text fig. 1, *1*), which soon become broad and somewhat flattened at the top. The calyx originates at an early stage (text fig. 1, *2*) as a marginal ring having five lobes. Considerable development of the calyx takes place before the differentiation of other floral structures, enclosing the broad, flattened, and slightly convex receptacle in a low, conical cavity (text fig. 1, *3*). The petals and stamens appear to arise simultaneously as upright lobes from the margin of the receptacle (text fig. 1, *4*). The petals are much thinner than the stamens and grow more rapidly. Their growth is less upright than that of the calyx, and the margin becomes somewhat incurved so that the cavity which they inclose becomes quite broad and flat. The pistil arises as a circular ring of tissue within the circle of stamens (text fig. 1, *5*). Soon growth begins in the central part of the receptacle, forming the placenta (text fig. 1, *6*),

which grows upward somewhat more slowly than the wall of the carpel, to which it is attached at two or three points. The edges of the carpel become drawn together above the placenta and for a time there is an open

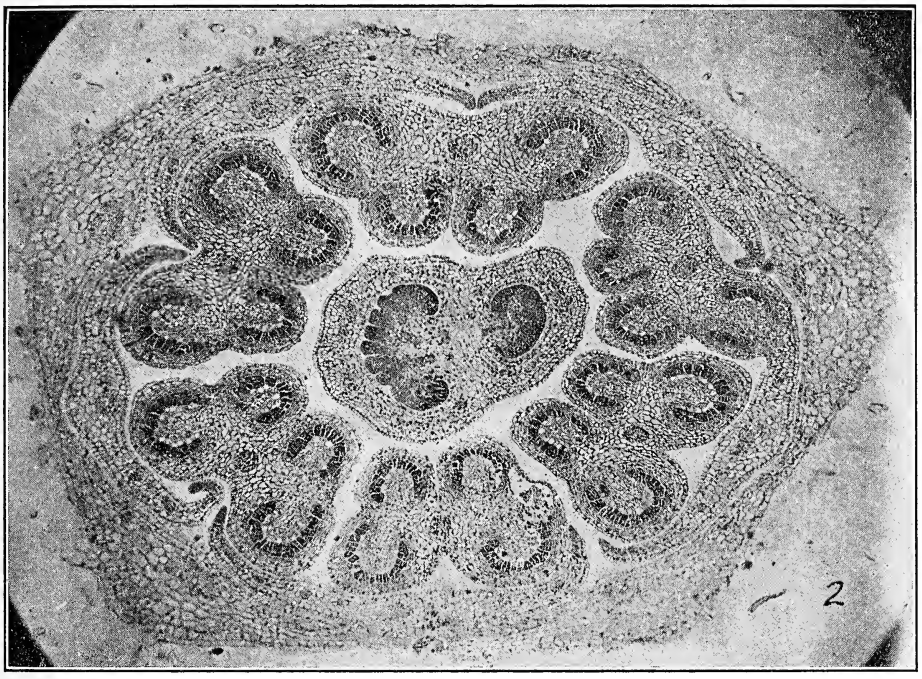


TEXT FIG. 1. Stages in the development of the flower of the potato (Beauty of Hebron). *R*, receptacle; *C*, calyx; *P*, petal; *St*, stamen; *Carp*, carpel; *Pl*, placenta. × 80.

canal through the style, but as the latter elongates the canal becomes closed with a delicate tissue of thin-walled cells.

A transverse section of the bud (text fig. 2) shows, on the outside, the calyx which is continuous except near the apex of the bud. The five

segments or lobes are distinguished as nearly triangular organs having a central strand of vascular tissue near the inner surface. The petals, five in number, alternate with the segments of the calyx. They are much thinner and flatter than the calyx lobes and are somewhat rolled inward at the margins. They have central vascular strands, also two much less conspicuous lateral strands. The stamens alternate with the petals, and the anthers in section are broad, four-lobed figures, each with a central vascular strand. The pistil is nearly round and typically consists of three carpels, though pistils with two or four carpels are not uncommon. Each



TEXT FIG. 2. Transverse section of bud of the Lookout Mountain potato. Note the horse-shoe-shaped masses of archesporial cells in the anther lobes. This section shows six anthers, one of which is borne on the edge of a petal. The ovary contains but two locules, and the beginning of ovule formation is shown by the lobed placenta. × 55.

carpel has a central vascular strand and incloses a cavity or locule which is nearly filled by the placenta. Later stages are characterized by the appearance of additional vascular tissue, especially in the placenta, where a strand is produced leading to each of the developing ovules. Cells containing crystal sand are quite common in the carpels, placenta, and calyx.

DEVELOPMENT OF THE POLLEN GRAINS

The sporogenous tissue appears in the anthers at an early stage. This tissue consists at first of a mass of small cells with dense, deeply-staining

contents and large nuclei, occupying each of the four lobes of the anthers. Soon the archesporium becomes differentiated (text fig. 2) as a layer about two cells in thickness, which is formed parallel to the surface of the anther and is covered by the epidermis and by a variable number of layers of parietal cells. The parietal tissue and the archesporium are derived from the same primary tissue of the anther, the sporogenous cells being distinguished by their denser and more deeply staining protoplasmic contents.

The archesporium (Pl. XXV, fig. 1, *A*) consists of a rather extensive tissue extending practically the whole length of the anther and appearing in the cross section as a horseshoe-shaped area very clearly marked off from the surrounding tissue. Since the anther does not split open at maturity but allows the pollen to escape by a terminal pore, the parietal cells show no modification of the walls to aid dehiscence. The tapetal cells (fig. 1, *T*) are rather large, active-appearing cells which, however, do not stain so deeply as the archesporial cells and which contain some vacuoles. The adjacent parietal cells gradually become flattened, and after the organization of the pollen grains the entire tapetum and the inner parietal layers become disorganized.

The nutritive cells on the inner side of the archesporial layer are very large and present the appearance of a glandular tissue (fig. 1, *N*). They constitute apparently the main source of supply of food material for the developing sporogenous tissue. The cell contents are less dense than those of the archesporium and contain large vacuoles distended with cell sap. Each cell at first contains a single nucleus, but by the time the pollen mother cells are developed most of the nuclei have divided, giving rise to large, binucleate cells.

The archesporial cells divide several times and the daughter cells remain in rather close contact, thus forming somewhat indefinite groups of cells extending, as a rule, transversely across the anther locule (Pl. XXV, fig. 2). The sporogenous cells finally increase considerably in size, lose their angular shape, and are then to be recognized as the microspore or pollen mother cells (Pl. XXV, fig. 3, *1*). These give rise to the pollen grains by two divisions in rapid succession. Before the cells divide, however, the cell contents become somewhat shrunken away from the cell wall. The first division is the heterotypic division in which the chromosome number is reduced to one half that found in the vegetative cells. The second is the homoeotypic division and gives rise to the microspores which develop into the pollen grains. Owing to the small size of the cells, it was not possible to make out clearly all the stages of chromosome behavior in the nucleus in the prophase of the heterotypic division, but there is no evidence that the behavior differs from that described in other species. The formation of a spireme, the synapsis stage (Pl. XXV, fig. 3, *2*), the loss of the nuclear membrane (fig. 3, *3*), and the separation of the chromatin material into chromosomes were observed, though the splitting of the spireme threads

and of the chromosomes could not be made out. Efforts to count the chromosomes were not very successful on account of their small size and crowded condition. It seems probable that the number of chromosomes in the somatic cells is either fourteen or sixteen. In the late stages of the heterotypic division, it is evident that the chromosomes have been reduced in number, and it is no doubt safe to conclude that from that time forward the germ cells contain one half the number of chromosomes found in the somatic cells.

After the loss of the nuclear membrane, a typical mitotic spindle is formed and the chromosomes come to lie in the equatorial plane of the spindle (fig. 3, *4*). The chromosomes pass quickly to the poles of the spindle, one half going to either pole (fig. 3, *5*). Here they assemble in a dense chromatic mass (fig. 3, *6*) which is soon relaxed and becomes surrounded by a nuclear membrane (fig. 3, *7*). The fibers of the spindle may be seen for a time between the daughter nuclei, but disappear before the homoeotypic division.

Only a short resting period intervenes between the heterotypic and the homoeotypic divisions. The nuclear membranes disappear, and the mitotic spindles are formed side by side in the cell (fig. 3, *8*). These lie at a considerable angle to each other, often crossing at right angles. In this division the chromosomes divide, each half passing to a different pole of the spindle (fig. 3, *9*). The daughter nuclei are developed and the spindle fibers persist for a time, but no cell wall is formed, all four nuclei lying free in the protoplasm of the cell (fig. 3, *10*). Soon constrictions appear in the surface of the protoplasm (fig. 3, *11*), which finally becomes separated into four equal parts each containing a nucleus (fig. 3, *12*). Owing to the tetrahedral arrangement by which one cell is usually more or less concealed from view, it frequently happens that only three cells are visible in a tetrad group.

All the pollen mother cells in a locule divide simultaneously. It is usual, however, to find the division stages somewhat more advanced at one end of the anther than at the other. The division occurs in other anthers in the same bud at nearly the same time, so that it is frequently possible to find all stages in a series of sections of the same bud. It occasionally happens that the homoeotypic division is omitted and that two cells only are formed from the mother cell (fig. 3, *13*). This occurs particularly in the ends of the locules.

The microspores of a tetrad are at first surrounded by the original walls of the pollen mother cells. These walls disintegrate and disappear as soon as new walls are formed around the microspores, which now become the pollen grains. These cells increase considerably in size and develop thick walls (Pl. XXVII, fig. 9). The further development of the pollen grains was not followed, as the material at hand failed to show the formation of generative and tube nuclei.

Development of the Ovule and Embryo Sac

The ovules are very numerous, completely covering the free surface of the placenta. They appear at first as erect, roundish protuberances (text fig. 2), but soon become somewhat pointed with the point directed laterally. Soon the integument appears as a relatively thick band of tissue closely surrounding the base of the central tissue or nucellus (Pl. XXV, fig. 4). The latter becomes obovate on account of the restriction of growth by the integument. The integument grows very rapidly and soon overtops the nucellus, which then ceases its growth; the single layer of cells of which the nucellus is composed becomes pressed flat against the inner surface of the integument and finally disappears. As the embryo sac approaches maturity, the inner cell layer of the integument becomes differentiated as a nutritive tissue, the cells becoming much enlarged and elongated in a direction perpendicular to the surface of the embryo sac (Pl. XXVI, fig. 6). This nutritive layer is continuous or nearly so with a group of similar but more angular cells in the chalaza, against which the embryo sac rests at its base. A single branch of vascular tissue from the bundles of the placenta passes upward through the funiculus and terminates in the ovule near the chalaza. The cells of the integument divide in all directions, producing an extensive tissue, and the embryo sac becomes deeply buried. The edges of the integument do not actually join over the end of the embryo sac, however, so that there remains a narrow and somewhat curved channel, the micropyle, through which the pollen tube may enter.

Meanwhile the lateral curvature of the ovule continues, and at length the opening of the micropyle is directed downward and is found not far from the funiculus. The ovule is not, however, of the typical anatropous type, since the embryo sac is considerably curved, suggesting a transition to the campylotropous form.

The archesporium may be distinguished at an early stage in the development of the ovule, usually in the form of a single hypodermal cell at the apex of the ovule. This cell is distinguished from the neighboring cells by its dense contents and deep staining reaction. It may not, when first discernible, be larger or have a more conspicuous nucleus than the surrounding cells. It grows rapidly, however, and the nucleus soon becomes quite large. At this stage it may be regarded as a megaspore; further development is delayed for a time (Pl. XXV, fig. 4).

As a rule, only a single archesporial cell is produced, though a number of ovules were observed containing two or more archesporial cells lying side by side, and in a single instance two such cells had developed to what may be regarded as the one-nucleate stage of the embryo sac. In nearly every case it appears that only one cell develops to the megaspore stage. Any other archesporial cells which may be present are crowded out and disappear at an early stage. In a few instances a row of two or three sporogenous cells was found in the axis of the ovule, suggesting a transverse

division of the original archesporial cell, though it is apparent that this is not the ordinary method of megaspore formation. It is very rare for the ovule to contain more than one mature embryo sac, yet three instances were found of what might be termed "double ovules." [1] In each of these cases, two embryo sacs were found enclosed by the integument at some distance from each other. Such a condition would arise only when the ovule is abnormal from an early stage in its development, having two distinct growing points with the archesporial cells separated by a considerable extent of nucellar tissue. Archesporial cells lying side by side would normally develop embryo sacs lying in close contact in the ovule.

The resting stage of the megaspore continues until about the time that the integument reaches the end of the nucellus and the pollen mother cells undergo the tetrad division. The megaspore, which has previously been somewhat angular, now elongates, its cell wall disappears, and its nucleus is somewhat enlarged. This is the one-nucleate stage of the embryo sac (Pl. XXVI, fig. 1). The nucleus then divides (Pl. XXVI, fig. 2), giving rise to the two-nucleate stage, one nucleus lying at each end of the embryo sac (Pl. XXVI, Fig. 3). Owing to the lack of cell-division stages in the ovules in the material examined, it was not possible to determine at what stage chromosome reduction takes place, though the chromosome number was found reduced at the first nuclear division in the embryo sac. Two more nuclear divisions now follow in quite rapid succession, giving rise to the four- and eight-nucleate stages of the embryo sac. Meanwhile the embryo sac has increased considerably in size and become deeply buried by the integument. The contents have become vacuolated, and four nuclei are present at each end of the sac.

The egg apparatus is now organized from the nuclei which lie in the end of the embryo sac nearest the micropyle (Pl. XXVI, fig. 6). Two of these nuclei, with the surrounding protoplasm, form the synergids. These are rather small, pear-shaped cells which develop acute points fitting into the end of the embryo sac close to the micropyle. The protoplasm in the basal portion of the synergids becomes very dense and stains deeply, so that the small nuclei can scarcely be seen. The upper part of each synergid is occupied by a single large vacuole and protrudes into the cavity of the embryo sac. The egg is placed laterally to the synergids and extends much farther into the embryo sac. It consists of a relatively large nucleus surrounded by dense protoplasm and has a large basal vacuole. Like the synergids, it is attached in the micropylar end of the embryo sac. The fourth or polar nucleus withdraws somewhat toward the center of the embryo sac, where it is joined by a second polar nucleus from the opposite end of the sac. These nuclei lie close together for a time but finally fuse to form the primary endosperm nucleus. Though the polar nuclei are scarcely as large as the egg nucleus, they are more clearly visible owing to the lesser

[1] Amer. Jour. Bot. 9: 213–214. 1922.

density of the surrounding protoplasm. The three cells remaining at the chalazal end of the embryo sac, the antipodal cells, are cut off by cleavage planes from the remainder of the embryo sac and stain very deeply. They are pressed against the chalaza by the growth of the embryo sac, become flattened, and finally disintegrate.

The fertilization of the egg and the development of the embryo were not followed, as these stages were not found in the material at hand. This material was collected in early summer from plants of the spring crop, at a time when weather conditions are generally unfavorable for the production of seed. It is accordingly not strange that stages in fertilization and embryo-development were lacking.

Degenerative Changes in Ovules and Pollen

The shedding of the potato blossoms and buds is preceded by degenerative changes which may be manifest to the naked eye or visible only upon microscopic examination. The macroscopic evidences are such as are ordinarily comprehended by the term "blasting." Growth ceases, the buds become yellowish, and wilting ensues. These symptoms are most noticeable when the buds are partly grown, and the changes involve both the anther contents and the ovules. When the pollen grains degenerate at a late stage of development, the ovules may be but slightly involved, many of them containing normal embryo sacs with eggs capable of being fertilized. In this case, normal blossoming may occur and viable seeds may be produced if weather conditions are favorable and if cross-pollination by a suitable variety is provided. This is the condition which obtains when sterility is due to hereditary causes acting principally upon the pollen. In such cases the anther locules are found to be empty, or contain the empty, shriveled walls of pollen grains. Under exceptionally favorable circumstances a few viable pollen grains may be produced, but there is little chance that they will reach the stigma of the blossom and cause fertilization, even if the anthers should not remain closed, as often happens in such cases. Sterility in this case is due to inherent lack of vitality in the pollen, the cells apparently being unable to continue their development after the formation of the thick walls cuts the grains off from their source of food supply.

Degenerative changes due to unfavorable conditions of the environment may have their inception at any stage in the development of the bud and usually involve the contents of the ovary as well as those of the anthers. The first indication of change in the archesporial cells is a starved appearance (Pl. XXVII, fig. 3). Growth and cell division cease. The nutritive cells and tapetum stain but lightly and appear to have but very scant contents. The archesporial cells become somewhat shrunken, the cytoplasm stains lightly, and numerous vacuoles appear, while the nuclei assume an abnormal, coarsely granular appearance. A little later the entire sporogenous tissue

collapses and passes into a state of disintegration. When the pollen mother cells are involved (Pl. XXVII, fig. 4), the cytoplasm shrinks away from the cell wall and appears as an elongated, shriveled mass within the cell, which takes a deep stain. The tapetal cells also collapse and the tissue disintegrates.

The period of tetrad-formation seems to be a critical time in the development of the pollen. Many instances were found in which degenerative changes had taken place at that stage. If the blasting occurs at the time of, or immediately following, the heterotypic division, the changes resemble somewhat those which take place in the pollen mother cells (Pl. XXVII, fig. 5). The cell contents become shrunken and stain deeply, and the daughter cells may become separated before disintegration takes place. If disintegration is delayed until after tetrads have been formed, a quite different series of changes is noted (Pl. XXVII, fig. 6). The tetrads remain in the original walls of the mother cells, and little or no shrinkage is evident. The cell contents stain lightly, numerous vacuoles appear, the cells at last lose their identity, and disintegration ensues.

It sometimes happens that no change is evident until after the walls of the pollen mother cells have disappeared and the cells of the tetrads have become rounded as pollen grains. Degeneration at this stage is marked by a cessation of growth and by the failure of the pollen grains to develop the characteristic thick coat (Pl. XXVII, fig. 7). The cell contents stain lightly and contain numerous vacuoles. Afterwards the entire cell breaks down and disintegrates. In case the thick coat has already formed around the pollen grain (Pl. XXVII, fig. 8), the latter does not reach the full size of the normal grain and is found at blossoming time either empty or containing a small amount of material in an advanced stage of disintegration.

Degenerative changes in the ovules generally accompany those in the anther, particularly where such changes take place in the early stages of pollen development. Change in the archesporial cells is characterized by cessation of growth and by a light staining reaction of the cell contents. The archesporial cells, however, do not disintegrate previous to the general breaking down of the ovules and placenta, which breaking down does not take place until the buds have dropped.

In the late megaspore stage or that of the one-nucleate embryo sac, when the integument has nearly covered the nucellus, blasting is accompanied by the shriveling of both the megaspore or embryo sac and the cells of the nucellus which surround it (Pl. XXVII, fig. 1). The changes which take place at later stages are quite similar, and there is nothing like the variety of changes which have been noted in the contents of the anther. The embryo sac and the remains of the surrounding nucellar tissue shrink away from the walls of the integument, stain deeply, and present a shriveled, disorganized appearance (Pl. XXVI, fig. 7, and Pl. XXVII, fig. 2). The shrinkage begins at the micropylar end of the embryo sac, the tissues remaining attached at

the chalaza. In the other tissues of the ovule, growth ceases and the cell contents display a somewhat lighter staining reaction than normally.

Changes in the ovaries are less likely to involve all germ cells than those which occur in the anthers. In the case of congenital changes which involve mainly the pollen and appear at a late stage in the development of the floral organs, many ovules may escape and develop into seed if the environmental conditions are favorable and if suitable cross-pollination is provided.

Summary

1. The potato is an unfavorable subject for cytological study on account of the small size of the cells and the degenerative changes which precede the shedding of the blossoms. These difficulties have deterred investigators from carrying out the study.

2. The development of the flower and of the germ cells does not show any marked differences from cases previously described for related plants.

3. Degenerative changes in the anther contents show decided differences according to the stage at which degeneration begins. Changes which occur at early stages of development appear to be due to unfavorable climatic or environmental conditions. The disintegration of nearly mature pollen grains appears to be the result of hereditary pollen sterility and does not interfere with the normal anthesis of the blossoms.

4. Degenerative changes in the ovules and embryo sac appear to result from unfavorable environment and may occur at any stage. They are much more uniform in character than those found in the anther.

5. Varieties which produce no viable pollen may set fruit and produce seed, provided environmental conditions are not such as to induce degenerative changes in the embryo sacs, and provided the blossoms are promptly crossed by a variety producing viable pollen.

EXPLANATION OF PLATES

Plate XXV

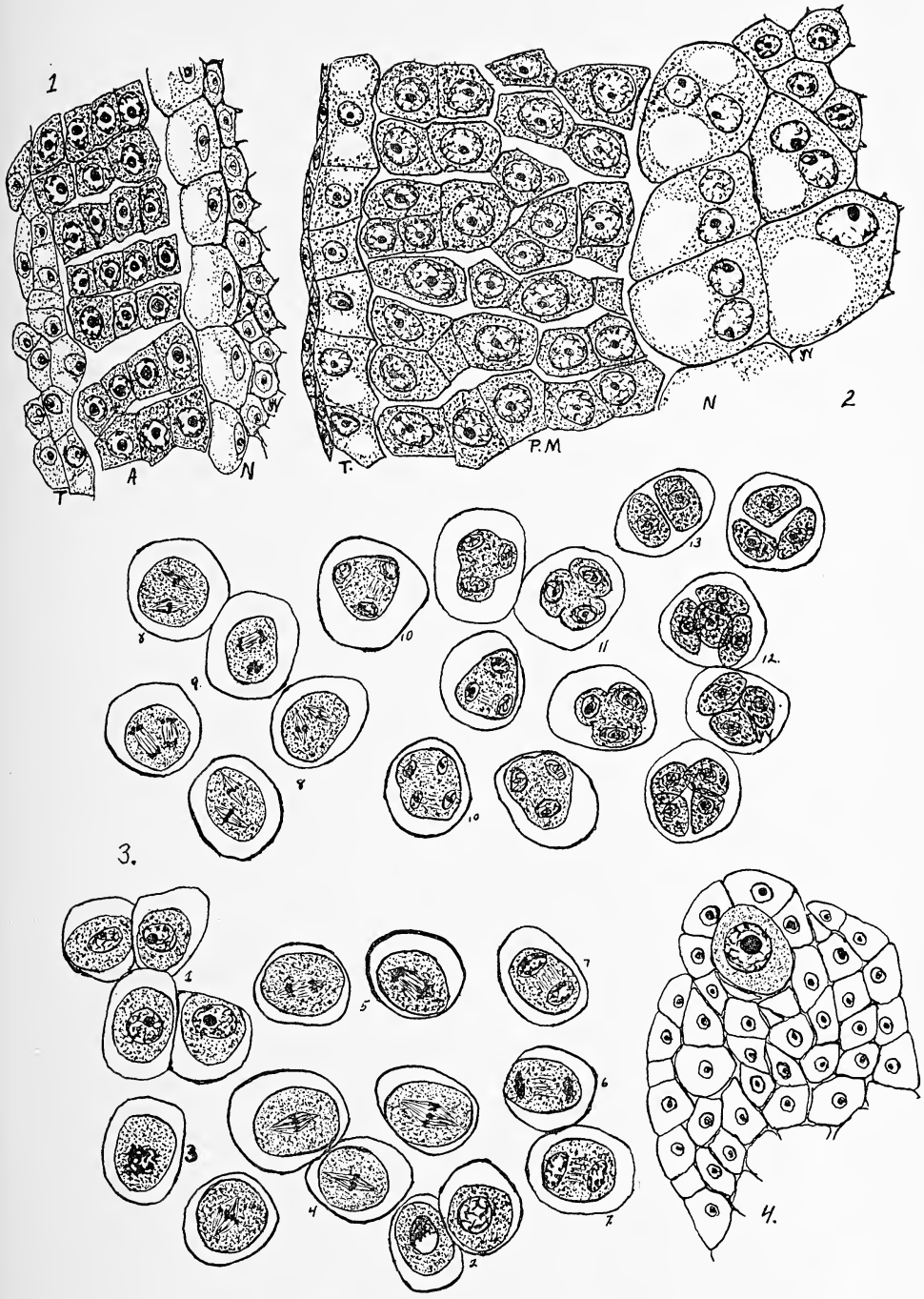
Fig. 1. *A*, Archesporial cells in anther. These cells are angular and arranged in groups. They have dense, deeply staining protoplasm and large nuclei. *T*, Tapetum. *N*, Nutritive cells. These tissues have to do with the nutrition of the sporogenous tissue and contain conspicuous vacuoles filled with cell sap.

Fig. 2. *P.M.*, Pollen mother cells developing from the archesporium. *T*, Tapetum. *N*, Nutritive cells, usually containing two nuclei each.

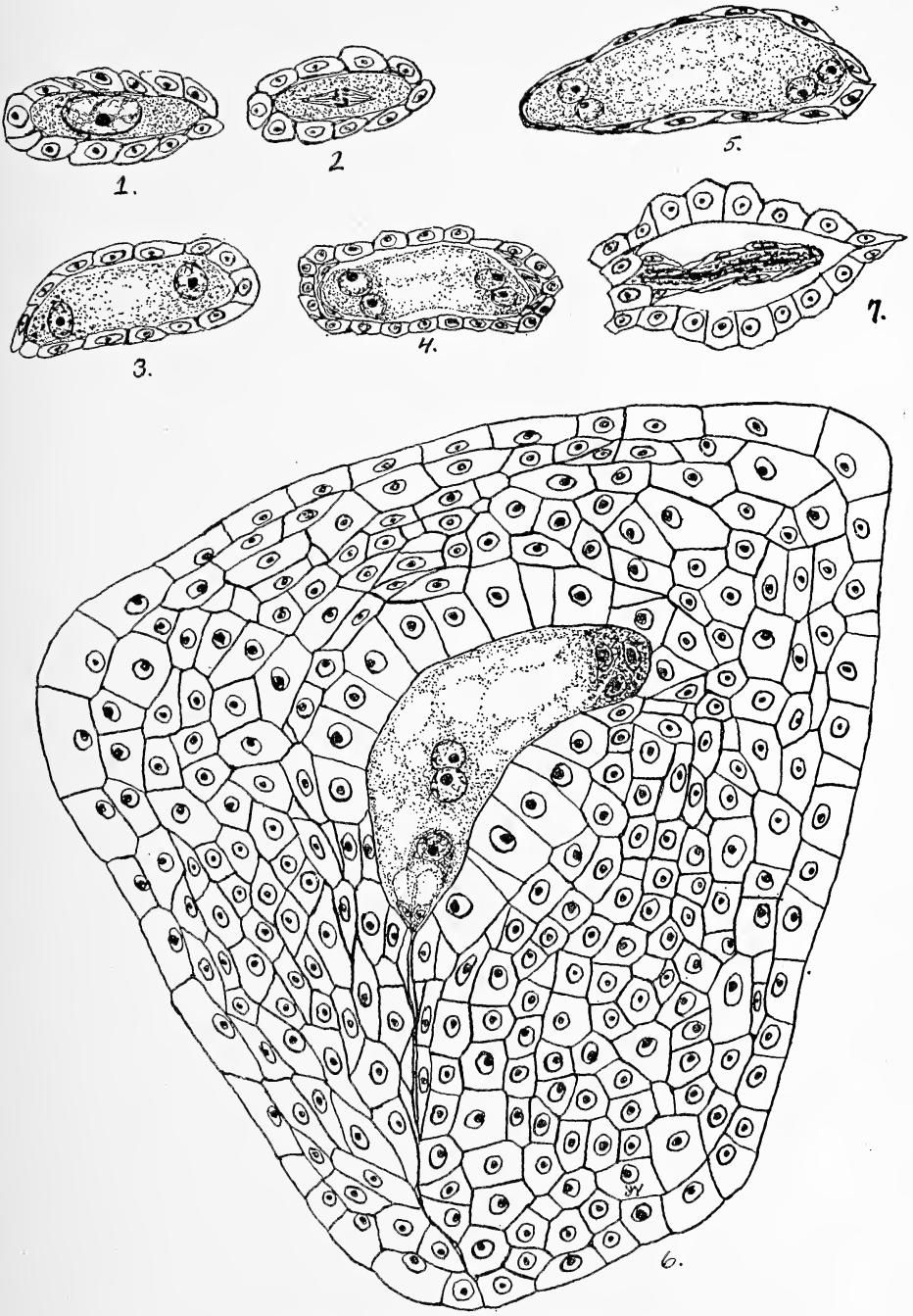
Fig. 3. Development of tetrads from pollen mother cells. *1*, Pollen mother cells. *2*, Spireme and synapsis stages. *3*, Prophase, nuclear membrane dissolved. *4*, Metaphase of heterotypic division. *5*, Anaphase of same. *6*, Telophase of same. *7*, Daughter cells of heterotypic division. *8*, Metaphase of homoeotypic division. *9*, Late anaphase or telophase of same. *10*, Daughter cells of homoeotypic division. *11*, Formation of constrictions which separate the microspores. *12*, Microspores formed but enclosed in original wall of pollen mother cell. *13*, Two cells formed by the heterotypic division, homoeotypic division omitted.

Fig. 4. Megaspore surrounded by the tissue of the ovule and the developing integument.

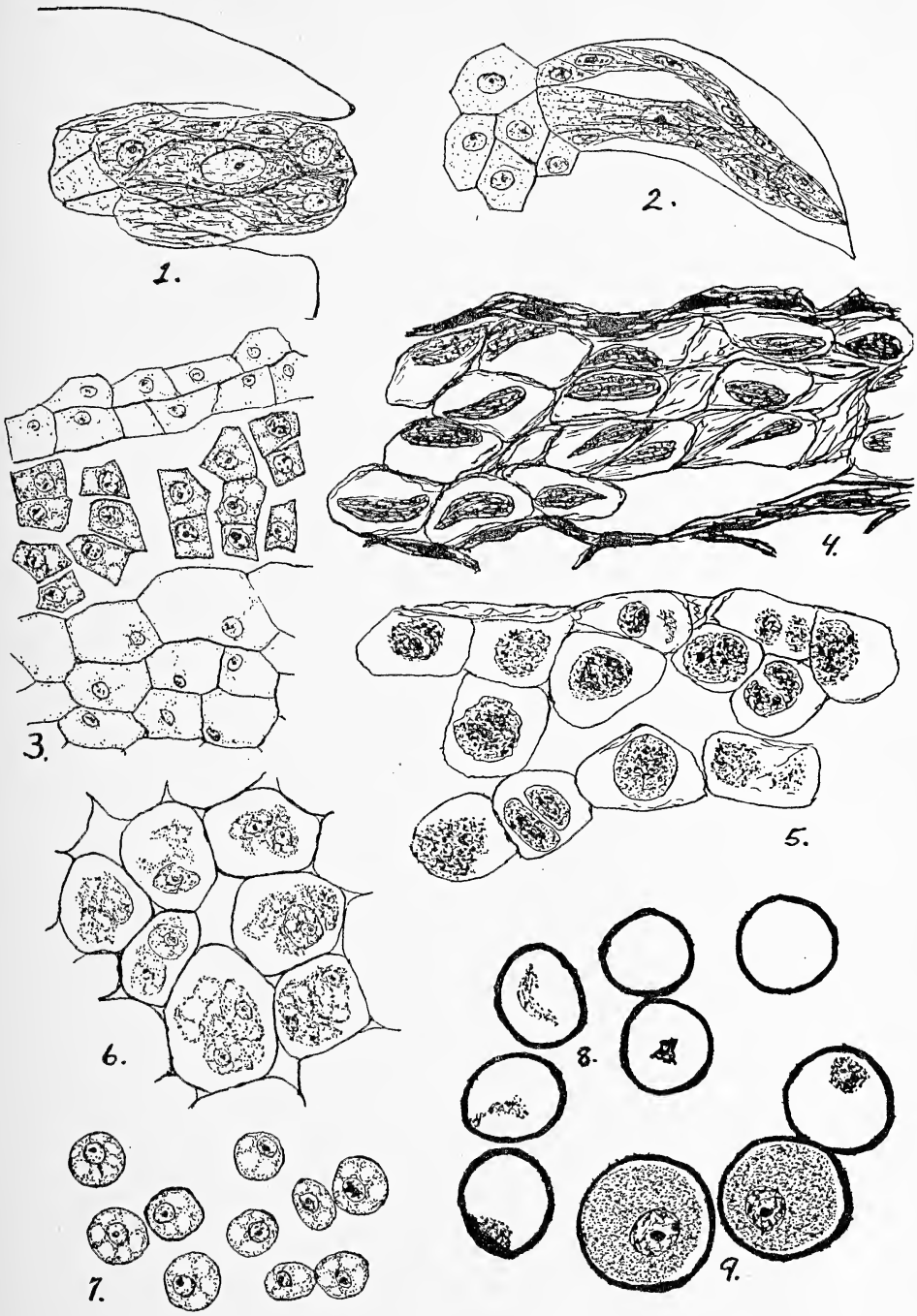
Lookout Mountain. × 680.



YOUNG: GERM CELLS IN THE POTATO



YOUNG : GERM CELLS IN THE POTATO



YOUNG: GERM CELLS IN THE POTATO

Plate XXVI

Fig. 1. Embryo sac, one-nucleate stage.

Fig. 2. First nuclear division in embryo sac.

Fig. 3. Embryo sac, two-nucleate stage.

Fig. 4. Embryo sac, four-nucleate stage.

Fig. 5. Embryo sac, four-nucleate stage just before the final division of the nuclei. The sac has grown to nearly its full size and has become slightly curved.

Fig. 6. Ovule containing fully developed embryo sac. At this stage the ovule consists almost entirely of the greatly thickened integument, the cells of which, surrounding the embryo sac, are enlarged and form a jacket of nutritive tissue. The integument does not close completely over the embryo sac. A narrow micropyle leads from the pointed end of the sac to the outside. The synergids are seen as small, pear-shaped cells in the micropylar end of the embryo sac, each having a large distal vacuole. The deeply staining egg lies close beside the synergids and has a large basal vacuole. The two polar nuclei lie side by side in the central portion of the embryo sac. These fuse later to form the primary endosperm nucleus. The three deeply staining antipodal cells occupy the base of the embryo sac.

Fig. 7. Disintegration of embryo sac.

Lookout Mountain. × 680.

Plate XXVII

Fig. 1. Disintegration of megaspore and nucellus. The integument is shown in outline.

Fig. 2. Disintegration of embryo sac at a later stage of development than that shown in Pl. XXVI, fig. 7.

Fig. 3. Early stage in degeneration of archesporial cells of anther.

Fig. 4. Degenerative changes in the pollen mother cells.

Fig. 5. Degenerative changes following heterotypic division.

Fig. 6. Degenerative changes following tetrad formation.

Fig. 7. Degeneration of young pollen grains.

Fig. 8. Degeneration of nearly mature pollen characteristic of congenital pollen sterility.

Fig. 9. Normal pollen grains previous to differentiation of generative and tube nuclei.

Figs. 1, 2, 8, and 9, Beauty of Hebron. Fig. 3, Early Ohio. Figs. 4 and 5, Dibble's Russet. Figs. 6 and 7, Mammoth Prolific. × 680.

THE EMBRYO OF LEMNA

FREDERICK H. BLODGETT

(Received for publication January 27, 1923)[1]

The writer was fortunate, several years ago, in locating a habitat in which Lemnaceae were abundant, with the special feature of regular flower-production in *L. perpusilla* Torr., which is there present. The development of normal flowers and mature fruits is the usual thing in this strain, rather than the partial development and later degeneration characteristic of *L. minor*, with which Caldwell worked.[2] The stages of development immediately following fertilization are in agreement with the description by Caldwell, and need not be restated; the following discussion deals with the formation of the embryo and germination of the seed, the earlier stages being in agreement with Caldwell's description in all essentials.

THE UNDIFFERENTIATED EMBRYO

The development of the embryo falls into three stages: (*a*) from the egg to the formation of a stem apex or equivalent; (*b*) development of the plumule and cotylar sheath; (*c*) formation of secondary structures in the plumule.

Reviewing briefly the early stages, the Lemna embryo develops without the formation of a proembryo, all the cells formed by the division of the egg remaining as part of the final structure. The embryo sac enlarges rapidly until it occupies the whole of the nucellus except a small cap of cells at the tip (Pl. XXVIII, fig. 1); and at the base a neck-like elongation is thrust into the chalazal tissues of the ovule. The endosperm cells formed in this chalazal neck quickly become dense in content, but the endosperm in other parts is less well developed, showing large cells with weakly staining contents during most of the period of development of the embryo. No suspensor is formed, the cells which first develop in contact with the inner surface of the embryo sac persisting throughout later stages, and not degenerating as do the cells of a suspensor when present. A secondary effect of fertilization in Lemna is the formation, from the tissues of the integuments immediately about the micropyle, of a structure best designated as an "operculum." This consists of cells greatly enlarged and with thicker walls than the rest of the integument, and at germination is thrust off as a cap by the elongation of the hypocotyl. As shown in figures 1, 2, 3, and 5,

[1] Published, at the expense of the author, out of the order determined by the date of receipt of the manuscript.

[2] Caldwell, O. W. Life history of *Lemna minor*. Bot. Gaz. **27**: 37–66. 1899.

Plate XXVIII, the inner cells of the operculum are approximately cubical in form, while those of the outer layer become elongated to resemble palisade cells in proportions, but are not of equal size in all parts of the cap.

Inception of the Stem Apex

At the stage when the position of the stem apex can first be located, the embryo is about twice as long as wide and broadly pyriform in shape. The apex is recognized by the appearance of a low mound of cells about midway of one side from base to tip of the embryo, which rapidly increases in size until nearly or quite hemispherical. The cells of this region differ in no evident way from those of the rest of the embryo. At this stage the embryo is 4 to 6 cells in diameter and from 12 to 15 cells in length. Figure 2 shows a stage slightly older, with the cotylar sheath making its appearance as a collar about the base of the apical mound. The anterior region of the embryo from this stage rapidly increases in diameter and carries with it the insertion of the apical mound, thus turning the axis of the mound through nearly 90 degrees, from a lateral protrusion to a position closely appressed to the hypocotyl. Meanwhile there is formed about the base of the apical mound a collar, which eventually completely encloses it, except for a pore left at the tip of the sheath so formed (*S*, fig. 4). From this stage it will be convenient to speak of the apical region as the "plumule," as it develops directly into the primary leaf structure, and of the enclosing wall as the "cotylar sheath," since it is formed from the tissues at the base of the cotyledon. The hypocotyl (*H*, fig. 4) is fused of course to the sheath tissues, and is indistinguishable from the rest except by position, and in sections such as shown. There is at no time a development of any structure resembling a radicle by the embryo in this region, and in the species studied there is none developed by the plumule, although in *L. minor*, as shown by Hegelmaier's[3] figures, a root is developed by the first leaf (plumule); this statement is copied in the recent paper by Goebel [4] and evidently confirmed by others for the same species.

The relation of parts in a well advanced embryo of Lemna is much the same as in an anatropous ovule, with the plumule taking the position of the nucellus, the cotylar sheath that of the integuments, the base of the cotyledon corresponding to the chalazal region, and the hypocotyl to the raphe of the ovule. In the fully grown seed, the plumule and cotylar sheath are nearly one half the full length of the embryo, the anterior half, or slightly more, of the embryo being true cotyledonary tissue and functioning at germination as an haustorial organ quite as definitely as does the scutellum in Gramineae, which latter has been compared to the "foot" of Selaginella

[3] Hegelmaier, F. Die Lemnaceen. Leipzig, 1868.

[4] Goebel, K. Zur Organographie der Lemnaceen. Flora **14**: 278–315. 1921. A discussion of views by Lotsy, Hegelmaier, and S. Rostowzew (Russian) on *L. minor*, with copied figures from three sources.

by Schlickum,[5] though he recognizes the difference in cell history. The similarity between the foot of Selaginella and the cotyledon of Lemna is most clearly seen in the germination stages, when the haustorial cotyledon remains within the testa as the sheath and the enclosed plumule protrudes from the seed and floats upon the surface of the water.

The Plumule

The stem apex develops directly into the plumule, or first leaf structure. It can not be separately identified either in the embryo or at later stages, and is recognized by the location of rudiments of leaf structures as projections from the meristematic tissue at the insertion of daughter outgrowths upon the parent tissues. This point, as relating to older individuals, was discussed in a previous paper.[6] The flattened character of the plumule is assumed at an early stage, as shown in figure 5, which is nearly intermediate in development between figure 2 and figure 3. A cross section at this stage would show a nearly circular embryo, with the plumule approximately lens-shaped and enclosed by the sheath tissues on all sides, but the walls of unequal thickness. No differentiation at this stage into further structures is noticed, but a little later the rudiment of the frond to be developed by the plumule as a daughter structure is apparent in sagittal sections, as at L^2, figure 4.

With the appearance of the rudiment of the second leaf the plumule becomes unequally lobed at the base, for the daughter frond develops on one side only in the plumule, as compared with the paired development of later stages. This is soon enclosed by a sheath from the base of the plumule in a manner quite like the ensheathing of the plumule by the cotylar pocket, and a similar shifting of direction of the axis of the enclosed structure takes place. Although Hegelmaier shows figures of germinating Lemna seeds with protruding roots as if from the plumule tissue, no indication of such development has been detected in the species under discussion, as a plumule characteristic. The first stage at which any root rudiment is discernible is after the daughter leaf (of the plumule) is well developed, and the seed nearly full-grown. At this stage a cross section would show structures as in figure 6. The root initials are just differentiating from parenchymatous tissue at the heavy line near the base of the daughter frond, L^2 of the key sketch. This occurs when the frond rudiment is about a dozen cells in length, but already nearly covered by the overgrowth of the pouch walls from the base of the plumule. The root initials are developed from cells in the second layer on the ventral side of the frond rudiment; the surface layer forms a sac about the elongating root in later stages, but this is finally pierced and then becomes the "root sheath" of the mature fronds. By

[5] Schlickum, A. Morphologischer und anatomischer Vergleich der Kotyledonen und ersten Laubblätter der Monokotyledonen. Bibliotheca Botanica **35**. 1896.

[6] Blodgett, F. H. Morphology of the Lemna frond. Bot. Gaz. **60**: 383–390. 1915.

the time the seed is fully mature, the root has become about twice as long as the diameter of its base, and forms a conical elongation quickly recognized as a root structure.

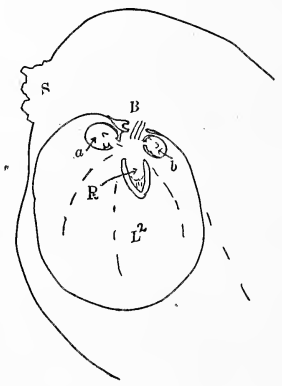
While the root initials of the daughter frond have been developing into the first root structure, the secondary frond-rudiments have been laid down at the base of the daughter frond, and are being enclosed by sheathing overgrowths from the adjacent parent tissue. This means that there are discernible in a seed prior to germination, in this species of Lemna, three generations of leaf structure: L^1, the plumule; L^2, its daughter frond, enclosed in the basal pouch; and L^3, the paired rudiments at the base of the daughter frond, each within its pouch (*a* and *b*, text fig. 1). By the time germination is under way, these also will have their daughter outgrowths laid down, and thus an additional generation of outgrowths will be present before the separation of seed coats from seedling is accomplished. But no definite apical region appears at any time; only the successive outgrowths show the presence of cells functioning as apical meristem in the region of insertion of the new fronds upon the older tissues, from which additional new outgrowths are put forth as if axillary to the last preceding structures in each pouch. In mature plants these additional outgrowths may be developed behind ("axillary" to) flowers, as well as behind vegetative fronds.

Germination

The ripe seed—or fruit, as the carpel usually persists about the seed itself—may be forced from the frond pocket by impact of rain or other surface disturbance, by the development of an outgrowth from the tissue at the base of the pouch as just mentioned, or, after the death of the frond, it may be released by decay. Under normal conditions the seeds may be found floating before germination is visibly begun, and at this time they lie at the surface upon their sides. Upon the actual inception of germination, the micropylar end of the seed becomes lighter, by virtue of the spongy character of the rapidly enlarging sheath tissue of the embryo, and this end comes uppermost, the seed now being vertical in the water. The operculum, previously mentioned as formed by the tips of the two integuments of the seed, is forced off, leaving the end of the seed open for almost its full diameter. Through this opening the sheath and enclosed plumule emerge, and the plumule promptly assumes its horizontal position on the water surface. At this stage the seed, protruding sheath, and emerging plumule together have the appearance of a diminutive railway spike, the seed forming the shaft, the sheath and partially protruded plumule the one-sided head, thick at the attachment and diminishing to a tongue-like margin.

The elongation of the tissues involved is chiefly by an expansion of existing cells rather than by a development of new ones, and this elongation is localized mainly in the region immediately adjacent to the insertion of the plumule upon the cotyledon or hypocotyl (fig. 7). The haustorial

cotyledon remains in the testa, and completely occupies the interior. The sheath tissues continue attached to the plumule as clasping lips upon the basal margin for a considerable time, in laboratory dishes until the daughter frond is well developed or even longer (but rain or other disturbances would act to separate these at an earlier stage). A seed, as it floats with the sheath tissues only protruding from the seed coats, resembles in size a normal specimen of Wolffia, but is of a different shade of green so as to be recognized quite readily in a mixture of the two. After protrusion the plumule expands rapidly, but remains enclosed at its base by the clasping sheath walls, as well as held by the tissues connecting hypocotyl and plumule, at the insertion of the latter upon the embryo structure. Daughter fronds appear promptly, and, as these protrude from the pouch of the plumule, they often force off the still adhering cotylar sheath, though in some cases this is not accomplished until the second fronds appear. The base of the plumule, when nearly fully expanded but before the daughter frond is protruded, appears as shown in text figure 1. In this figure, *S* indicates the point of attachment to the seed, *B* is the base of the daughter-frond stem, inserted upon plumule tissue; L^2, the daughter frond, with *R*, its root rudiment. The secondary fronds are shown at *a* and *b*, nearly or quite enclosed in pouches; and on the short stipe behind the base of L^2 may be noted a minute rudiment of an "axillary" bud, arising from the meristem of the plumule, as if from apical tissue. The rudiments, *a* and *b*, in the daughter frond arise in a similar manner, but appear to be differently situated through the thrusting forward of the region upon which they are inserted, by an elongation of the stipe and the backward overgrowth of the frond pocket.



Text Fig. 1. Base of plumule, with daughter frond (L^2) in place, root rudiment at *R*, paired rudiments of next fronds at *a* and *b*; stipe of daughter frond inserted upon plumule at *B*; former attachment to cotylar sheath at *S*. Young frond rudiment as axillary bud on side of stipe near *B* (axillary to L^2). On same scale as figure 7, Plate XXVIII.

Flowering Plants

In the strain under discussion, the tendency of the plants to produce flowers is so strong that flowers have been found upon specimens still retaining the adherent seed coats and the clasping sheath of the cotyledon. These flowers had come as the second generation of outgrowths from the plumule, or as the equivalent to L^3 in frond sequence. It is to be noted that in these cases, as in more mature specimens, the flowers are produced from the pouch in the reëntrant angle formed by the fronds, rather than projecting from the outer and protruding angle at their junction. This would preclude any possibility of floating contact between plants as a means of pollen transfer, as two flowers would be separated by the combined distance of their respective angles, which distance approximates the length of the frond itself. The stigma is elevated above the surface in such a manner as to avoid contact with the surface so far as possible, so that floating pollen is not a likely means of transfer from stamen to pistil. Further, the pollen grains are spinose, as in many insect-carried forms, which fact would indicate the possibility of insect assistance here also. In the habitat concerned, there are a number of species of Thysanura, and other minute insects in considerable abundance. The actual assistance of these in the pollination of Lemna has not as yet been proven, but their presence among the plants, and the spinose character of the pollen, are at least suggestive of insect aid, and may explain the regularity of seed-formation in this locality. This point is still under investigation as opportunity offers, but it was not thought wise to defer indefinitely the publication of other results in respect to the sporophyte of *Lemna perpusilla*.

Summary

The embryo of Lemna develops without the formation of suspensor cells or proembryo; all the cells formed from the egg evidently going to the formation of the true embryo. No radicle is formed, even in rudiment, by this species, and the cotyledon is terminal and massive.

The stem apex appears as a lateral mound of cells about midlength of the embryo when 12–15 cells in length, and develops directly into the first leaf, or plumule.

The plumule elongates at the same time that it is being enclosed by an overgrowth backward from the base of the cotyledon, and is turned into a position parallel to the hypocotyl.

When fully developed, the plumule is obliquely cordate at base, flattened lens-shaped, and completely enclosed by the cotylar sheath; the tip of the plumule and the apex of the enclosing sheath lie just under the micropyle.

A daughter frond is early formed in the larger basal lobe of the plumule, and this develops the first root structure formed in this species.

In basal pouches of the daughter frond, paired frond rudiments are

initiated at an early stage of germination, or even in the fully mature embryo prior to actual germination.

The plumule is protruded from the cotylar sheath at germination in such a way as to lie upon the water surface, the attached seed and ensheathing embryo adhering to the base of the plumule for some time, the cotyledon acting haustorially in the testa.

Flowers are produced freely in this species in the habitat examined, and the presence of minute insects suggests the possibility of insect-pollination.

Flower-formation may be begun by plants with only two or three leaves from the seedling, when developed in laboratory dishes.

State Normal School,
Danbury, Conn.

EXPLANATION OF PLATE XXVIII

Drawings made by aid of an Edinger apparatus; figures 1–6 uniform in magnification with the scale shown; figure 7 on smaller scale, as shown.

Fig. 1. Longitudinal section of carpel and ovule; embryo shaded. Tip region of integument tissues changing into "operculum" about micropyle. Axillary bud behind carpel at *B*.

Figs. 2, 2*a*. Longitudinal and cross sections of young embryo, showing early stage of development of plumule (stem apex), and inception of sheath about base of plumule. Operculum of two forms of cells, short from the inner integument, elongated from the outer layer.

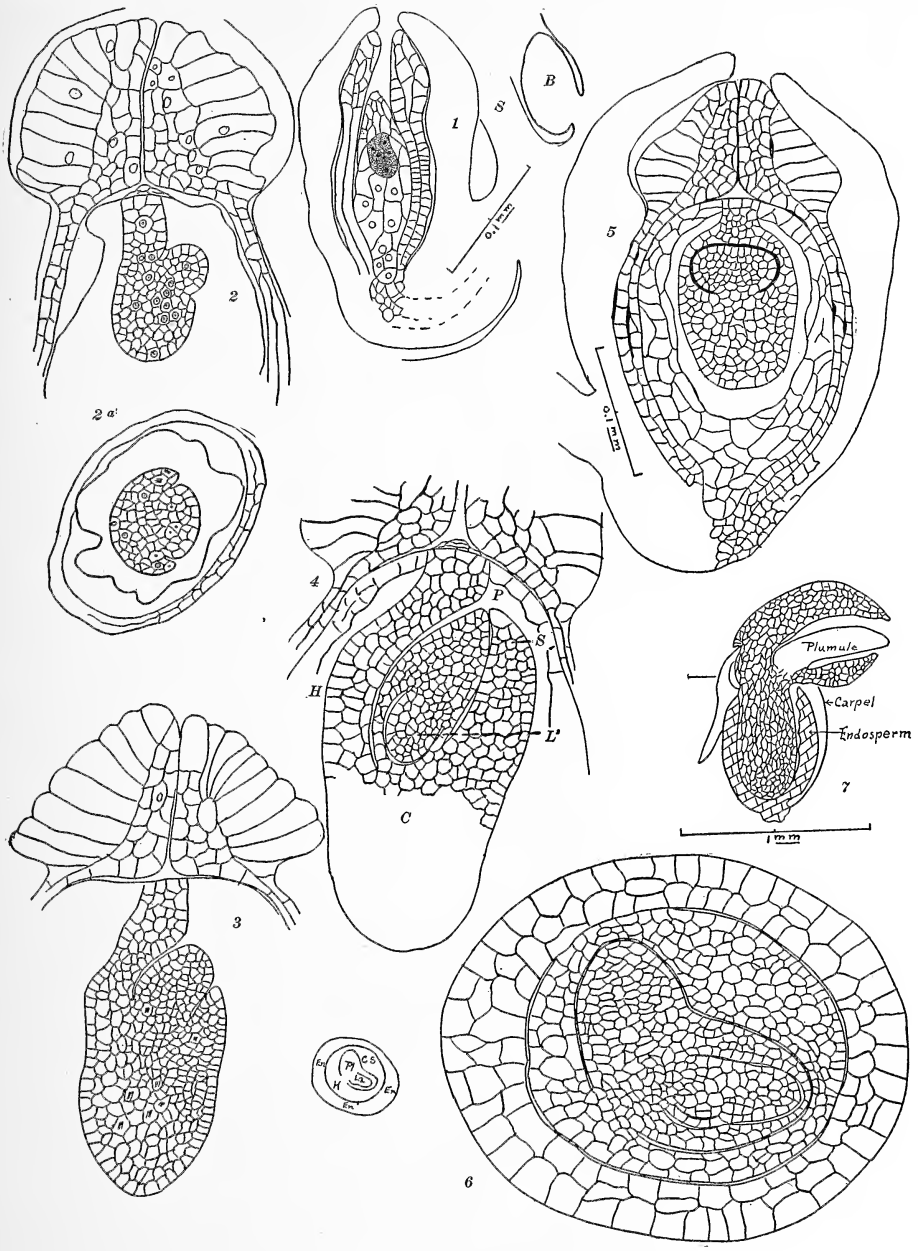
Fig. 3. Embryo in longitudinal section, showing plumule half enclosed by sheath, hypocotyl attached to embryo sac without change in character of cells. Cotyledon terminal.

Fig. 4. Plumule nearly covered by sheath, rudiment of daughter frond present in pouch at base of plumule. *H*, hypocotyl; *S*, tip of sheath; *P*, tip of plumule; L^2, daughter frond.

Fig. 5. Section at right angles to last, but at a little earlier stage, showing the plumule as a flattened lobe in upper half of embryo. Age of embryo just prior to that of embryo shown in figure 3.

Fig. 6. Cross section of nearly mature seed with parts identified by key sketch (at left). *En*, endosperm; *H*, hypocotyl; *CS*, cotylar sheath; *Pl*, plumule; L^2, daughter frond.

Fig. 7. Sagittal section of germinating seed, cotylar sheath with enclosed plumule protruded from seed coats, cotyledon retained in testa as "foot." Endosperm diagrammatic, water surface indicated by horizontal line, plumule tissue (fine-celled and deeply staining) in outline.



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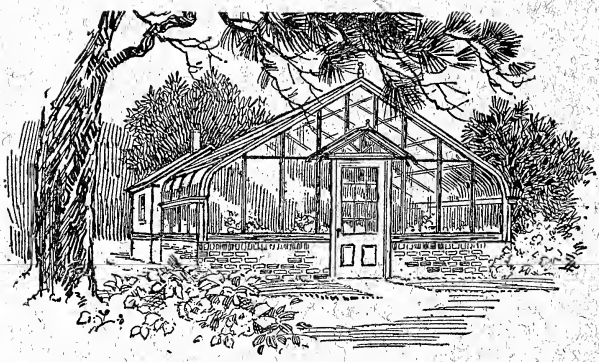

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THE STRUCTURE OF THE CHROMOSOMES IN *TRADESCANTIA VIRGINICA* L.

HAROLD C. SANDS

(Received for publication October 19, 1922)

As a result of studies on the nuclei of *Tradescantia virginica* L., I am taking up previously discussed questions relating to the structure of the chromosome.

That there is still little agreement in regard to this important problem is shown by the relatively recent papers of Bonnevie (1908), von Herwerden (1910), Nawaschin (1911), Erhard (1910), Vejdovsky (1912), Lundegårdh (1912*a*), Suessenguth (1921), and Martens (1922).

An examination of the literature discloses a great volume of data bearing on this important subject, which perhaps originated with the much quoted paper of Balbiani (1881), in which he first called attention to striations on the chromatic filaments of the nucleus.

Throughout this mass of literature, figures are given which represent appearances obtained by different methods of fixing, staining, and imbedding. As noted, the interpretations of these microscopic appearances in the chromosome vary widely, and are frequently influenced too much by attempts to harmonize the descriptions of them with what is believed to be true regarding their behavior.

I shall restrict my description to certain stages: in the pollen mother cells, those from diakinesis (Häcker, 1905), to the arrangement of the chromosomes on the equatorial plate (*Sternfigur* of Flemming) just prior to separation; and further, both in the pollen mother cells and the somatic cells, to the stages after complete separation has been effected, *i.e.*, from the beginning of the anaphase to that telophase stage just prior to the reformation of the daughter nuclear membranes. I shall omit the series of processes involving the separation of the chromosomes during the metaphases.

The more intimate structure of the chromatic *Fäden* or *Schleifen* has been much discussed for every point between these stages, but, in following the older authors, it is frequently difficult to know to which particular stage of the division figure the writer refers. Naturally, such ambiguities

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in the older literature are to be expected, for at the time, the steps in mitosis were not so specifically defined. In this description, reference is made to the individual chromosome and not to tetrads as such.

My material was prepared by the modernized aceto-carmine method as described by Belling (1921*b*). Material was also fixed in Flemming's medium fluid followed by sectioning and staining. In addition to these methods, pollen mother cells were teased out on a slide into a 3 percent cane-sugar solution and studied in the living condition. The staminate hairs were suspended in 3 percent cane sugar, and the course of the divisions could be followed as described by Strasburger (Practicum, p. 604) for the staminate hairs of Tradescantia and by Lundegårdh (1912*b*) for root-tip divisions. The temperature was satisfactory when between 75° and 80° F.

The drawings of Plate XXIX were made entirely from aceto-carmine preparations. This is certainly a most valuable reagent for studying the chromatic elements of a cell to the exclusion of others. The solution may be employed in any dilution, and I found that for general purposes one drop of the modernized Schweigger-Seidel (1868) preparation added to one drop of water on a slide gave almost instantaneous staining.

The fluid acts as a swelling agent, so that the preparations in a one-to-one dilution go to pieces in about a week. For this reason, it is desirable to know how soon after treatment the chromosomes will show any particular stage in the swelling.

The mother cells were teased from the anthers into a drop of water on a slide to which then was added, ordinarily, a drop of the aceto-carmine. The mount was made as described by Belling (1921*b*), who first instructed me in the method. It was noted, in cases in which the stain was run under the cover glass, that about ten seconds were required to bring the chromosomes into the sharpest definition.

The structure came out with the first swelling, somewhat as does the image on a photographic plate, without any perceptible change either in the form or in the position of the elements from that seen in the living condition. The first perceptible change can be noticed in about an hour, when the elements may or may not appear a trifle swollen. When slight swelling occurs, it affords considerable advantage for studying the morphological composition of the chromosomes.

The figures given on the accompanying plates were, for the most part, made shortly after fixation, *i.e.*, after some slight swelling had occurred. The effect with the reagent noted, while apparently altering the indices of refraction of the cell elements and thus increasing the sharpness of their differentiation, leaves the chromosomes, especially, quite unaltered except for the probable slight hydration.

Most of the currently used killing agents, and especially the practice of hardening and imbedding, tend to greater density and even shrinkage of the cell structures. The use of such a fixative as this is especially advantageous as a check and for comparison.

The structures here are more or less obscured and are, in fact, rarely seen in the material prepared by the standard killing solutions and imbedding methods. A comparison of Tradescantia prepared by these two methods convinces me that, in the sectioned material, the finer details of the living cells are lost by slight fusions, shrinkage, and distortions, which leave perhaps the relative proportions as a whole unimpaired.

By dilution technique, any desired degree of staining intensity may be obtained ranging from none or the natural hyaline to that of deep color. As stated, the most frequently used dilution was, perhaps, one drop of aceto-carmine to one drop of water. The rate of disintegration suffered by the mounts prepared with this dilution was usually so rapid that one could not study the preparation for several consecutive days. Weaker mixtures were therefore devised.

Dilutions were made in a series of test tubes, so that tube *A* contained a one-to-one mixture; tube *B*, a one-in-four; tube *C*, a one-in-eight; tube *D*, a one-in-sixteen; and tube *E*, a one-in-thirty-two. If the tubes are cleaned of all foreign inorganic and organic matter, the stain will not precipitate and the tubes may be kept corked. Paraffined corks should be prepared, otherwise evaporation may be considerable. For most anthers, tube *E* contained too much water in proportion to acetic acid so that the effects of toxic stimulus generally appeared.

This method was found preferable to one in which pipettes graduated to hundredths of a cubic centimeter were employed for making the dilutions directly on the slide. Too much inaccuracy resulted from the latter practice.

The 1:16 dilution was very satisfactory, and permitted one to observe the cells unstained for a long period of time, then through all intermediate stages of slow color absorption till at last the chromatin contents are of a deep rose color. This latter may require anywhere from six to twenty hours, since the contents of the anther sacs do not all react alike to the staining fluid. Some take up the stain more slowly than others. I found that with weaker dilutions the cells would often burst; in order to overcome this the dilutions were made more nearly isotonic by the addition of cane-sugar solution instead of water.

The dividing pollen mother cells of *Tradescantia virginica* L. treated as above described show clearly that the chromatic elements are composed of bodies roughly similar to those described by Balbiani (1881) and by Pfitzner (1882). The shape and size of these granules and their arrangement or distribution in the chromosome have not, however, been adequately described.

In figures 11 and 13, Plate XXIX, some of the strands are seen to be apparently made up of two rows of granules side by side. By numerous authors, these granules have been spoken of as *chromomeres*, a term introduced by Fol (1891). Eisen (1900) distinguishes *chromioles* as components of chromomeres. I also consider the bodies making up the chromosome

to be compound, but do not find a definite number of the bodies that he defines as chromioles. O. Hertwig (1906) applied the term *chromiole* to the *Scheibchen* of Chironomus nuclear filaments.

The shape of the chromomeres varies from spherical to oval, to cubical, with or without rounded edges and corners, and finally to irregular. As generally found, they are *doubtless aggregates* and are made up of smaller elements which again may be found subdivided into varying grades of smaller particles. It has been generally held (Mottier, 1907, p. 336; Miyake, 1905; and others) that these chromomeres are not the ultimate units of the chromatin itself. Strasburger says:

> Eine Ide ist wie wir sahen, nicht der letzte der im Kern festzustellenden Strucktur-elemente; sie geht aus der Vereinigung kleiner Gebilde hervor (1905, p. 53).

Conversely, then, as I consider it, the ultimate chromatic particles agglutinate into groups, these unite to form an aggregate of groups, and the group aggregates unite to form a chromomere. The whole process may be a series of successive agglutinations which involve, in the first instance, the migration of the minute particles through the homogeneous matrix (*Zwischensubstanz*) of the chromosome, *i.e.*, the linin.

The term *Id* has been avoided because it is not desired to associate these bodies with Weismann's (1885) conceptions, nor indeed is it intended to identify the chromomeres as described with any of the theoretical loci recently developed in the genetical field (see Mottier, 1907, p. 336).

I consider the unit of these agglomerates to be the ultimate microscopic chromatin particle as observed usually in *fixed material*, and as discussed by Wilson (1900, p. 37). Meyer (1920) has recently formulated the most specific hypothesis yet advanced as to the ultimate ultra-microscopic composition of the chromatin and cell contents. He assumes as essential constituents of the cell at least three types of so-called *Vitüle*, cytoplasmic vitules, nuclear vitules, and trophoplasmic vitules. These vitules are also extremely complex and are assumed to be made up of so-called *mions*. The mass of an electron is said to be 2,000 times smaller than that of a hydrogen atom. Meyer assumes the mion to be 2,000 times smaller than the electron. I shall take up this point in another paper.

In the mother cells, the linin appears to be distinct in composition from the linin of the achromatic spindle, since, in preparations stained with aceto-carmine, it appears rather dense and of a most delicate straw to rose color, whereas no element of the achromatic spindle is either stained or perceptible. On the other hand, in preparations of the stamen hair, the elements of the achromatic spindle are clearly visible both in stained and in unstained material but only between anaphase and telophase. The surfaces of the *Zwischensubstanz* are clearly marked, not only by a very delicate color differentiation, but also by their relative refractive indices.

How many grades in size there may be in the subdivision of the chromomere is not clear. Figure 8, Plate XXIX, figures the construction as seen

in the aceto-carmine preparations. Here only two size differences could be clearly seen, and this represents the most that could be counted.

Strasburger (1904) assumes a compound construction for his *gamosomes* as he does for the *Ide*, but not quite in the same sense as presented in this paper. He says:

> Das Chromatin zieht sich aus den Lininfäden zurück und lässt sie als wenig tingierbare, zarte, perlschnurartig gegliederte Fäden zurück. Es bildet Körnchen, die sich um einzelne Zentren sammeln.

The chromomeres of figure 8, Plate XXIX, seem to be made up of four smaller bodies each, but this is of course an optical section. Careful focusing up and down shows others to lie beneath. I have seen these smaller bodies again subdivided, but this is not always a mathematically regular phenomenon, such as Miss Merriman (1904) has reported for Allium. I am convinced of the accuracy of her figures 35, 39, and 41, Plate XII, but do not feel that she is warranted, without further evidence, in drawing the conclusion that each subdivision of the chromosome proceeds by fours any more than Bonnevie (1908) would be justified in such a statement by reason of her figures 11, 16, and 22, Plate XIV.

The number of these subdivisions is variable. A careful study shows that the chromatic thread, contrary to the impression first given as it is seen in figure 13, Plate XXIX, is not made up of chromomeres side by side. The arrangement is more complex than this. In any given field of vision and with any group of chromosomes, the breadth of the individual chromosome, as well as that of any length of thread exclusive of constriction points, is constant, which would not be the case if they were made up of rows of bodies side by side, that is, if their real shape was ribbon-like.

Heidenhain (1907) figures chromosomes which appear ribbon-like in cross section (text fig. 1). I have not seen such forms either in the living material or in that fixed by any of the methods mentioned above.

In figures 3 and 4, Plate XXIX, an enlarged drawing of the ends of two threads is seen. Here one sees that the chromomeres are apparently arranged irregularly just inside the periphery of the linin cylinder. The surface of the cylinder may be nodular, irregular, or smooth, according to whether or not the chromomeres extend beyond the periphery.

The chromomeres shown in figure 10 project more than those in figure 5, so that the degree of irregularity presented by the chromosomal outline is a function of the degree of projection of these bodies. In the unstained and living chromosome, the surface has a punctate appearance, not only in the telophases but in the early anaphases or even before, so that, while Lundegårdh (1912*b*) has described a nodular chromosome structure in the telophases of the vegetative divisions, I would extend this description to include the reduction divisions and, in all cases, the chromosomes of the metaphases and anaphases. Some evidence of this is seen in figure 15, Plate XXX. Note the last chromosome at the bottom of the figure.

This photograph was taken from a stamen hair suspended in 3 percent cane-sugar solution and unstained. The chromosomes are living, and numerous ones appear quite smooth. It was found that the greater the density of the sugar solution, the greater was the apparent smoothness of the chromosome outlines.

The central axis of the chromosome remains clear of these bodies. One may say that its structure, in this sense, is tubular. To use a homely comparison, if one could fill a sausage skin with irregular pebbles in such a manner that the central axis would remain clear, and if the pebbles pressed against the skin so as to give it an irregular nodular outline, this would represent crudely the structure of the chromosome. The bounding membrane would, of course, represent merely the linin surface, and a truer description would perhaps regard the chromomeres as more or less imbedded in a linin cylinder as a continuous phase. Figure 19, Plate XXX, also from a living unstained preparation, shows, in the upper half, the ends of two chromosomes. In these two, the hollow structure is quite obvious even in the photograph. It was, naturally, much more so to the eye of the observer.

An outer chromosomal membrane such as Wenrich (1916) describes for Phrynottetix has never been observed by me in Tradescantia. Such a membrane could be demonstrated neither in the living chromosome in a sugar solution nor in any of the fixed material. For the present, I prefer to consider such figures as Lee's figure 4, Plate I, (1920) to be due to the action of fixing fluid which caused the chromatin to shrink, thereby leaving the achromatic substratum through which it was originally distributed unchanged in form. His vacuolated condition of the plant chromosome as distinguished from the solid structure of the animal chromosome must also be due to the fickleness of our preparation methods.

In figure 9, Plate XXIX, the chromosomes are about to be oriented on the equatorial plate. One observes here several whose longitudinal axes lie parallel with the optical axis of the microscope so that the ends are presented to the eye of the observer (*b*, fig. 9).

The relationship of the chromomeres, one to the other, is somewhat variable. Sometimes they show traces of a spiral arrangement (figs. 17, 18, Pl. XXX). At other times they are mostly paired, as in figure 16, or else irregularly spaced as in figures 11 and 13, Plate XXIX. In figure 12, chromomeres 2 and 3, 5 and 7, 10 and 12, 13 and 14, are located in the median optical plane of the chromosome and possibly opposite each other, so that the free spaces taken along the chromosomal axis represent the distances between them. The chromomeres 4, 6, 8, 11, and 15 of this figure overlie the hollow center and more or less overlie the other chromomeres.

This figure represents a chromosome that became freed from its mother cell. Part of the cell wall had been torn by the needle while being teased

from the anther sac, but when the cell was found the metaphase-plate figure was still in position and intact. By pressing successively on the cover glass with a fine, springy needle, the chromosomes were finally separated from the cytoplasm. By proper manipulations, often by merely tapping the microscope stage with a pencil, the specimens would roll over, thus permitting the observer to view all sides of the chromosomes. The chromosome in question was studied in this way, and it was then discovered that the chromomeres numbered 4, 6, 8, 11, and 15 were opposite others separated from them by the hollow center of the chromosome. The whole chromosome was found thus to have a rather regular four-rowed construction. (See Nawaschin, 1911.) That this is perhaps not a general condition is shown by the arrangement in figure 1, Plate XXIX, where chromomeres 4, 7, 8, and 20 seemed to be arranged in various positions with respect to the others. Here, again, the arrangement of those in the median optical plane of the chromosome appeared opposite.

In figure 2, the chromomeres have been numbered with reference to their distance from the eye-piece as determined by focusing up and down through the mass of the chromosome. Chromomere no. 1 is nearest to the eye; no. 14, the farthest from the eye. Those between are in successively intermediate positions.

It is considered that in figure 12, chromomere 16 is at the distal[1] end of the chromosome, *i.e.*, at the end away from the spindle attachment. Chromomeres 2, 5, 8, and 10 are therefore higher than 3, 7, 9, and 12. In the same figure, chromomeres 4, 6, 8, and 11 seem to have the planes of their sides somewhat oblique to the long axis of the chromosome. The arrangement as a whole simulates a segmented spiral, which is also the case in figure 5, notably, here, between chromomeres 10, 11, 12, and 13. On the other hand, one arguing for the view that the chromomeres are parts of fragmented disks and hence opposite will find, as noted, some evidence to support such a claim.

An understanding of the true construction of the chromosome is obviously very dependent upon a study of the segments in all possible optical planes. Let text figure 2 represent the cross section of a chromosome when the construction is assumed to consist of four rows of chromomeres arranged opposite to one another, and the line *po* the median optical plane. If the focus is adjusted to the line *xy*, the image of the chromosome will show numerous, practically solid, cross striations or bands. It was by carefully focusing up and down that chromomere *c*, figure 9, Plate XXIX, and the overlying chromomeres in figure 1 of the same plate were located. In the latter case, the specimen was rotated on the microscope stage by tapping, and its cross section while on end was studied. The band-like structure is seen here and there in figure 10, Plate XXIX, while in figure 9 the two-ranked chromomeres, as well as a band in a few cases, are seen, accord-

[1] Wenrich (1916).

ing to the focal depth at which the bodies are observed. (Compare the photographs on Plate XXX.)

This composite construction of the chromosome holds true for the chromosomes of the homoeotypic divisions. These chromosomes appear more gracefully modeled than do those of the heterotypic spindle. Figure 5 is a free-hand drawing of one of the second-division chromosomes, and is the same one as *a*, figure 6, Plate XXIX. The relations of the chromomeres seem to be the same here as already discussed, but they can be made out only under the most favorable conditions.

Figure 7 represents two free-hand drawings of the same chromosome. The upper drawing is of the top focus; the lower is at the bottom focus. From these drawings one obtains an idea of the irregularly shaped chromomeres that are sometimes encountered. One is able to trace the shapes of the bodies through the linin mass from top to bottom. The clear space or constriction in the center resulted from applying pressure to the cover glass while teasing the chromosomes into the suspension fluid, so that too much importance may not be attached to forms such as these.

The linin substratum seems to be of a jelly-like consistency, and if the chromosomes are crushed the chromomeres often fuse in a more or less solid mass. Varying degrees of contact from complete independence to complete fusion may be observed in cells injured by dissection instruments.

A Correlation of these Findings with Others of a Similar Nature

Balbiani (1881) first called attention to disks of chromatin in the nuclear filaments of salivary-gland cells in Chironomus larvae. These had been fixed in a mixture of $\frac{1}{2}$ percent chromic- and acetic-acid solutions. The glands were treated with the reagent for a few minutes, washed in distilled water, and, after staining with methyl green, were mounted in glycerin.

A considerable amount of work on the same material has been done, principally by Lydig (1883), Carnoy (1884), Korschelt (1884), Erhard (1910), von Herwerden (1910), Bolsius (1911), and Alverdes (1912). All the authors agree on the compound nature of the threads but differ as to the details.

Balbiani considered these chromosome constituents to be disks arranged in a series with clear spaces filled with *Zwischensubstanz* separating them. He likens them to a series of red blood corpuscles placed end to end.

Lydig (1883) held that the dark transverse segments of the chromosome are themselves made up of smaller bodies, and that the lines separating these may make up something like longitudinal lines of division in the chromatic material. It may be noted that these more irregular lines of division agree in part with my findings as shown by several figures of Plates XXIX and XXX.

Korschelt held that the cross and other markings are due merely to the wrinkling of the chromosome surface.

Henle (1882) held that the striations seen by Balbiani and others were artifacts and the result of post-mortem coagulation, but Carnoy (1884) took exception to his contention:

> On peut affirmer que le boyau de nucléine existe pendant la vie, tel que nous venons de le décrire. C'est en effet sur les cellules vivantes que nous l'avons étudié, fig. 66 et 67. Les boyaux si volumineux des insectes sont faciles à examiner de cette façon. On les voit parfaitement, même avec l'objectif DD, à l'intérieur du noyau; on peut y suivre les circonvolutions, et y distinguer nettement les stries dont nous parlerons tout-à-l'heure.

Objections may arise upon comparing these gland-cell nuclei with other nuclei involved in ordinary mitotic divisions, since, in connection with secretory activity, it is common to observe nuclei of irregular constitution and shapes such as those in the spinning cells of Arachnids—the spireme nuclei of Wilson (1900, p. 35)—or those found in the polymorphonuclear leucocytes. The structures under discussion are not confined to these cells. Carnoy (1884) described them for the plant *Paris quadrifolia* simultaneously with other forms (text fig. 3).

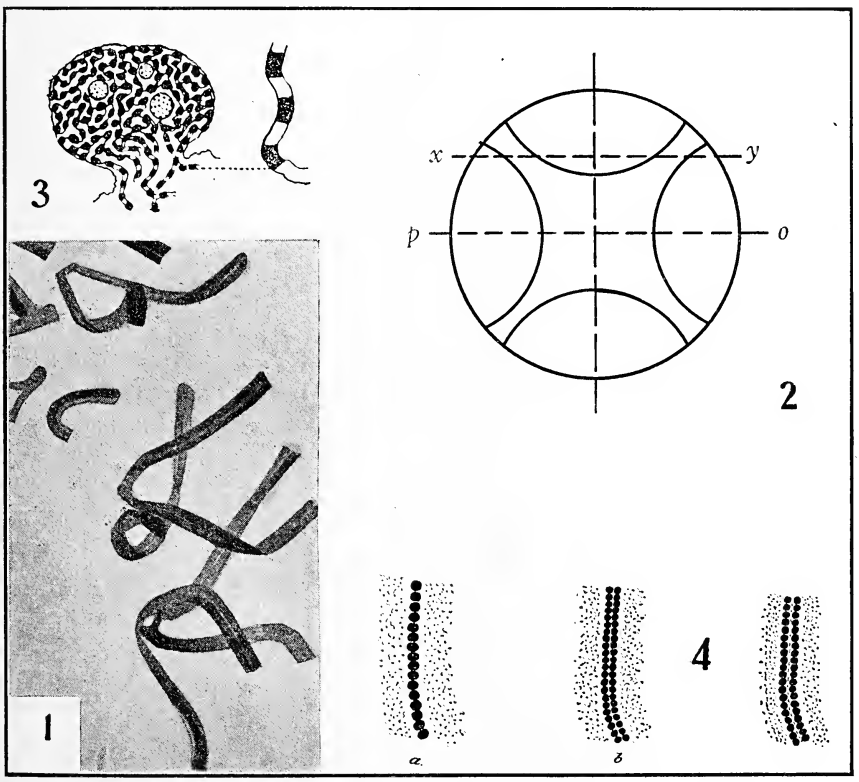

Fig. 1. Ribbon-like chromosomes figured by Heidenhain (1907). Fig. 2. Schematic representation of a quadripartite chromosomal cross section. Fig. 3. Chromomere construction as figured by Carnoy (1884) in *Paris quadrifolia*. Fig. 4. Pfitzner's conception of the splitting chromatic granules, taken from Flemming (1882).

Baranetzky (1880) first studied chromosome structure in *Tradescantia virginica* L. He notes that:

> Die Schärfe und die Regelmässigkeit des Baues ist oft so gross, dass beim ersten Anblick die Kernfäden unwillkürlich an die aus platten Zellen bestehenden Oscillarienfäden erinnern.

He further notes that the contours of the chromosome outlines are finely wavy owing to a slight constriction of the *Zwischensubstanz* between the disks. He describes the splitting of the chromosomal disks as taking place at right angles to the long axis of the chromosomes, and further states that the cleft begins at the surface of the disk and proceeds toward the center without good evidence that he has actually observed the process. Baranetzky also reports the same structure of the chromosomes for Agapanthus, Hemerocallis, Yucca, Hesperis, Lathyrus, and Pisum.

Erhard's (1910) aberrant ideas as to the relation of the chromatin and the nucleus need hardly be considered.

Pfitzner (1882) described the chromatic threads of the nuclei of many different tissues as being made up of granules, the "Pfitzner granules" (text fig. 4).

It is to be emphasized that there is practically no disagreement on the point that this construction obtains for the prophase stages. One of the first to point this out was Hermann in 1891. Almost every investigator has figured the chromatin bodies at this stage, notably Flemming (1879), Strasburger (1882), Bonnevie (1908), Vejdovsky (1912), and others too numerous to mention.

Flemming (1882), working with fixed material, recognized the granular composition of the chromosomes throughout the process of division (text fig. 5). His figures of the prophases, equatorial plate, anaphases, and telophases show an almost diagrammatic regularity in the arrangement of the chromatic granules. He explains the granular structure of the longitudinal halves of the chromosomes in the monaster by ascribing *the apparent loss of chromosomal continuity* to the same effect of fixing reagents which causes, in the earlier stages, the obliteration of the split in the chromatic filaments.

Strasburger (1884), working on Tradescantia, recognizes the Pfitzner granules as microsome disks and the *Zwischensubstanz* as hyaloplasm. He also claims, for the early prophase, that the material of the chromosomes may be in the form of a slender spiral. In his figures 63, 64, and 65 *a*, *b*, *d*, Plate XIV, he clearly shows the constricted surface outlines of the chromosomes in the metaphases, and in figure 49 of the same plate he shows it in the anaphases.

Miss King (1901), working on *Bufo lentiginosus*, finds the chromosomes of the ovum to be composed of microsomes.

Vejdovsky (1907) discusses the granular construction of the chromosome as described by Flemming. He says that young chromosomes do not consist of a homogeneous substance, but show a pale substratum on

whose surface are disposed fine granules separated at regular intervals. By treatment with the EH method, these granules acquire the deep black coloration of the earlier stages while the ground substance appears yet paler. According to him, the granules may be looked upon as chromomeres. On page 21 of the same paper he says that the *constricted chromosomes*, as they are figured by most authors, especially by Van Beneden, Herla, and Bonnevie, consist of independent knots or rings of chromatic substance in the ground material which corresponds to the original *Lininsubstanz*. The chromosomes appear here as if segmented, and the individual seg-

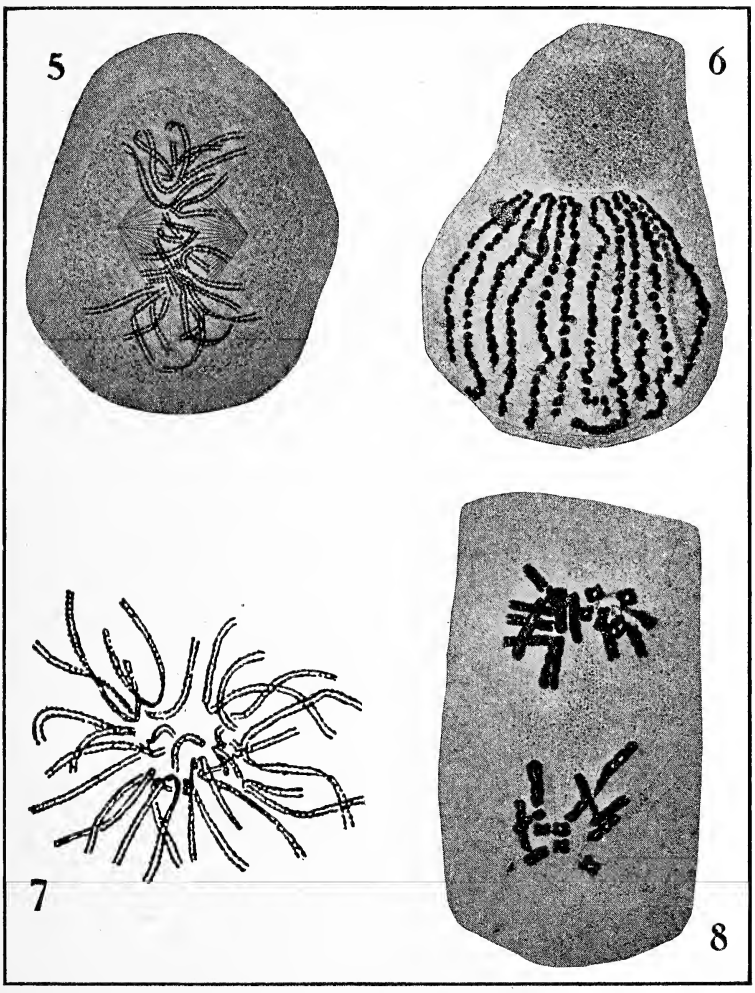

FIG. 5. Chromomeres figured in the metaphases by Flemming (1882). Salamandra. FIG. 6. Chromomeres in the prophases, figured by Hermann (1891). FIG. 7. Metaphase chromosomes of Salamandra showing granular composition, and assumed by Flemming (1882) to be longitudinally divided. FIG. 8. End views of chromosomes which show hollow and quadripartite structure. Merriman (1904).

ments of chromatic substance remind one of the chromioles of Eisen. Vejdovsky shows in his figure 164, *a*, *b*, *c*, *d*, *e*, *f*, and *g*, Plate IX, the metaphase chromomeres in Rhynchelmis and Enchytraeus. It is to be noted that this figure presents a striking resemblance to figure 38, *g*, Plate 3, of Wenrich's (1916) publication.

Vejdovsky says, further, that the slender halves of the split chromosomes consist of chromomeres bound together by delicate, slightly stainable bridges. He calls attention to the fact that the same structure was seen in the earlier stages of chromosome formation in Enchytraeus and assumes that, since they appear again later, their loss during the intermediate stages is due simply to the contraction of the chromosomes.

Later, in 1912, Vejdovsky describes the so-called *chromonema* which, in my opinion, is merely a very accurately described fixation distortion. His 1907 publication was, it seems to me, a more accurate presentation of the facts. One serious objection to the spiral structure was that it permitted no simple assumption as to the means for effecting the longitudinal splitting of the chromosomes. Vejdovsky avoids this difficulty by explaining, on page 21, that the spiral becomes altered, at metaphase, to groups of chromatic bodies in the nature of chromomeres which take up positions opposite one another so as to permit an equal longitudinal fission of the chromosome. (See his paper of 1912, Plate III, figures 42 and 43).

As I have already stated, I feel that Miss Merriman (1904) (text fig. 8) is not justified in extending her hypothesis of the quadripartite structure of the chromosomes to all the subdivisions that may occur, but it is interesting to note the persistence with which the structure shown by my text figure 2 is given by investigators. I refer to Bonnevie (1908), figures 7, 11, 19, and especially 22, Plate XI (text fig. 9), and in particular, to those of Nawaschin (1911) (text fig. 10), and Chambers (1915).

Von Herwerden (1910), working on Chironomus, believes the chromosome structure is spiral, but, on account of the granules she observed on them, she concludes by associating the latter with the ids of Weismann.

Schustow (1913) in his figures 4 and 6, Plate XIV (text fig. 11), and figures 29–33, Plate XV, shows the anaphase chromosomes to be hollow structures. Compare these figures with those of Bonnevie's (1908) figures 8, 10, and 22, Plate XI; 56, Plate XIII; and 75 and 79, Plate XIV. Although these figures of Bonnevie and Schustow do not especially show the chromomeres, they do illustrate the fact that the stainable material of the chromosome is peripherally placed, at least at these stages. The assertion of Schustow that the cross section of the early anaphase chromosome is circular whereas that of the late anaphase is polygonal has, I believe, no significance with reference to a telophase longitudinal split.

In discussing the distribution of the chromatin in the chromosome, Carnoy (1885) says:

> A l'intérieur de ce tube se trouve la nucléine, ou chromatine des auteurs. La manière

dont celle-ci s'y présente est variable. Ici elle remplit entièrement son étui; là, dans les boyaux volumineux, elle se retire contre la paroi, en laissant ouvert un canal central renfermant un plasma transparent.

Terni (1914) has, in many figures, very diagrammatic chromomeres. I consider his splitting chromosomes to be a misinterpretation of a chromatically hollow structure.

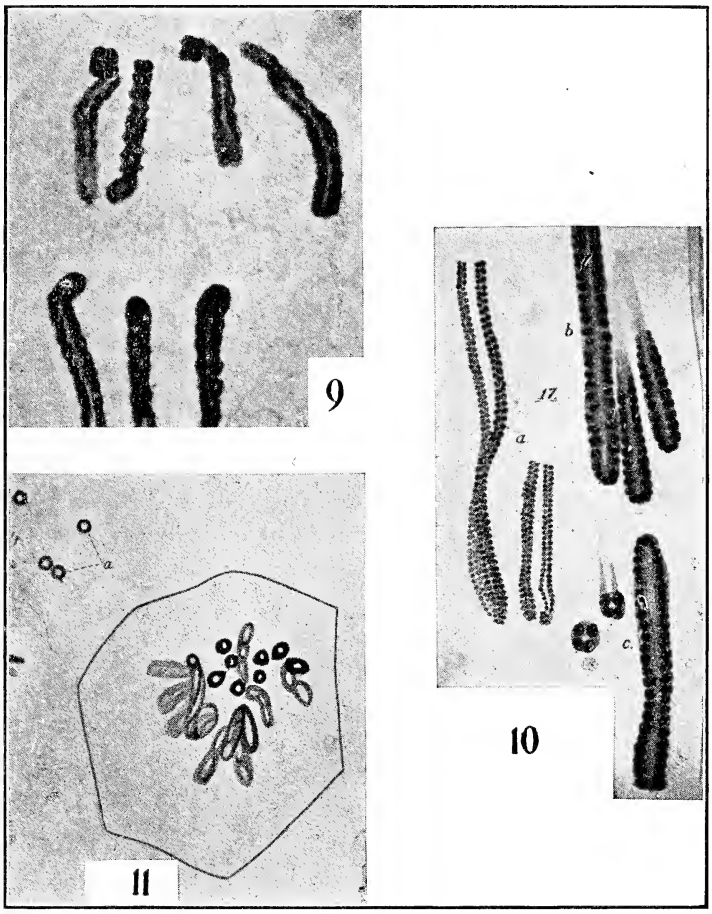

FIG. 9. Quadripartite chromosomes of Ascaris. Bonnevie (1908). FIG. 10. Chromosome end views showing quadripartite structure and chromomeres. Nawaschin (1911). FIG. 11. Chromosome end views showing chromatin-free centers. Schustow (1913).

Chambers (1915) working on *Disosteira*, describes a hollow chromosome structure in the living condition. He finds that the chromomeres are arranged in such a manner that there results a central achromatic core.

Wenrich (1916, p. 117) says, in speaking of Vejdovsky's 1912 publication:

I find little evidence of a *chromonema* in the telophase of the spermogonial divisions and what evidence there is would indicate that the chromatin becomes distributed on the inner surface of the vesicular walls, not on the outer surface of an achromatic core.

There is an implication here that the chromosome is hollow in somewhat the same sense as I have described it.

According to Suessenguth (1921), who worked on Rhoeo, one of the Commelinaceae, the chromosome has a moniliform structure made up of ten segments. Each of the latter, I take it, would be homologous to the chromomere as defined by Eisen. Eisen considered that the chromomere is made up of six chromioles, but that the number of chromomeres in a chromosome may be variable.

In conclusion, it seems to me, both from my own work and from the evidence in the literature, that the distinction between chromatin and linin is universally valid, and that, when preparations do not show this distinction, as in those of Grégoire and Wygaerts (1903) and of Sharp (1913, 1920) for the telophases, where, according to them, the chromosomes break up by vacuolation, it is due either to overstaining or to shrinkage.

The linin of the chromosome is distinctly a more or less regular cylinder of jelly-like consistency in which the chromatin is imbedded. It is not provided with an outer membrane which could be demonstrated by any of the methods used.

The chromatin is grouped into bodies, the generally recognized chromomeres. In Tradescantia these are of variable shapes and sizes, and, as far as present evidence goes, their number in the chromosome seems variable.

The colloidal structure of the chromosome is conceived to be primarily a two-phase system with the chromatin representing the disperse phase and the linin, the continuous phase. The continuous phase, within the body of the chromomere, may be more dehydrated than that present in the linin proper. That there may be still further phases of colloidal dispersion within the chromomere is not denied. At any particular stage of mitosis, an individual chromomere may have a greater or a lesser degree of dehydration than its neighbors in the same or in other chromosomes. This *degree of dehydration* may represent a specific constant for each chromomere for any particular stage. Since the coefficient of dehydration between the chromomeres may be a variable, only identical phases would be comparable, and it might be expected that in the *end result* of fixation, imbedding, and staining, the relative proportional dehydration values would be maintained.

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EXPLANATION OF FIGURES

Plate XXIX

All the figures of this plate are from the chromosomes of *Tradescantia virginica* L. Figures 1, 2, 3, 4, 5, 7, 8, and 12 are freehand. Figures 6, 9, 10, 11, and 13 are drawn with the camera lucida at table height (1600 diameters), with 2 mm. Zeiss apochromatic objective N.A. 1.4 with 10x compensation ocular, at 160 mm. tube-length and with critical illumination.

Fig. 1. Portion of a metaphase chromosome teased from a partially injured cell into the suspension fluid.

Fig. 2. Portion of a metaphase chromosome from the same cell.

Fig. 3. Enlarged end views of two separate chromosomes from different cells.

Fig. 4. Enlarged end view of another chromosome.

Fig. 5. Chromosome *a*, figure 6. The numbers are given for convenience in referring to individual chromomeres.

Fig. 6. Chromosomes of a pollen mother cell in the homoeotypic division. The lower dotted line represents the outline of the nucleus belonging to the other sister spindle which completed its dispireme in advance of the one drawn.

Fig. 7. A chromosome of the equatorial plate, first division, teased from the same cell as were those of figures 1 and 2. It was slightly damaged in the process, and I ascribe the constriction to the teasing. The upper figure shows the appearance at the top focus; the lower one is from the deeper focus.

Fig. 8. Chromosomes of the equatorial plate, first division, showing subdivision of the chromomeres. Taken from a perfect cell and a common figure.

Fig. 9. Metaphase chromosomes of the first division.

Fig. 10. Metaphase chromosomes with marked nodular outlines.

Fig. 11. Late prophase or diakinesis just prior to the equatorial plate stage.

FIG. 12. Chromosome from the same cell as those shown in figures 1, 2, and 7.

FIG. 13. Chromosomes in diakinesis.

PLATE XXX

The photographs were taken with a Zeiss apparatus, 2 mm. apochromatic objective, N.A. 1.4, compensation ocular no. 4, bellows 50 cm. Arc and color screens. × 1500.

FIG. 14. Metaphase (somatic) in the staminate hair of Tradescantia stained by aceto-carmine 1 : 4. The chromomeres are quite distinguishable.

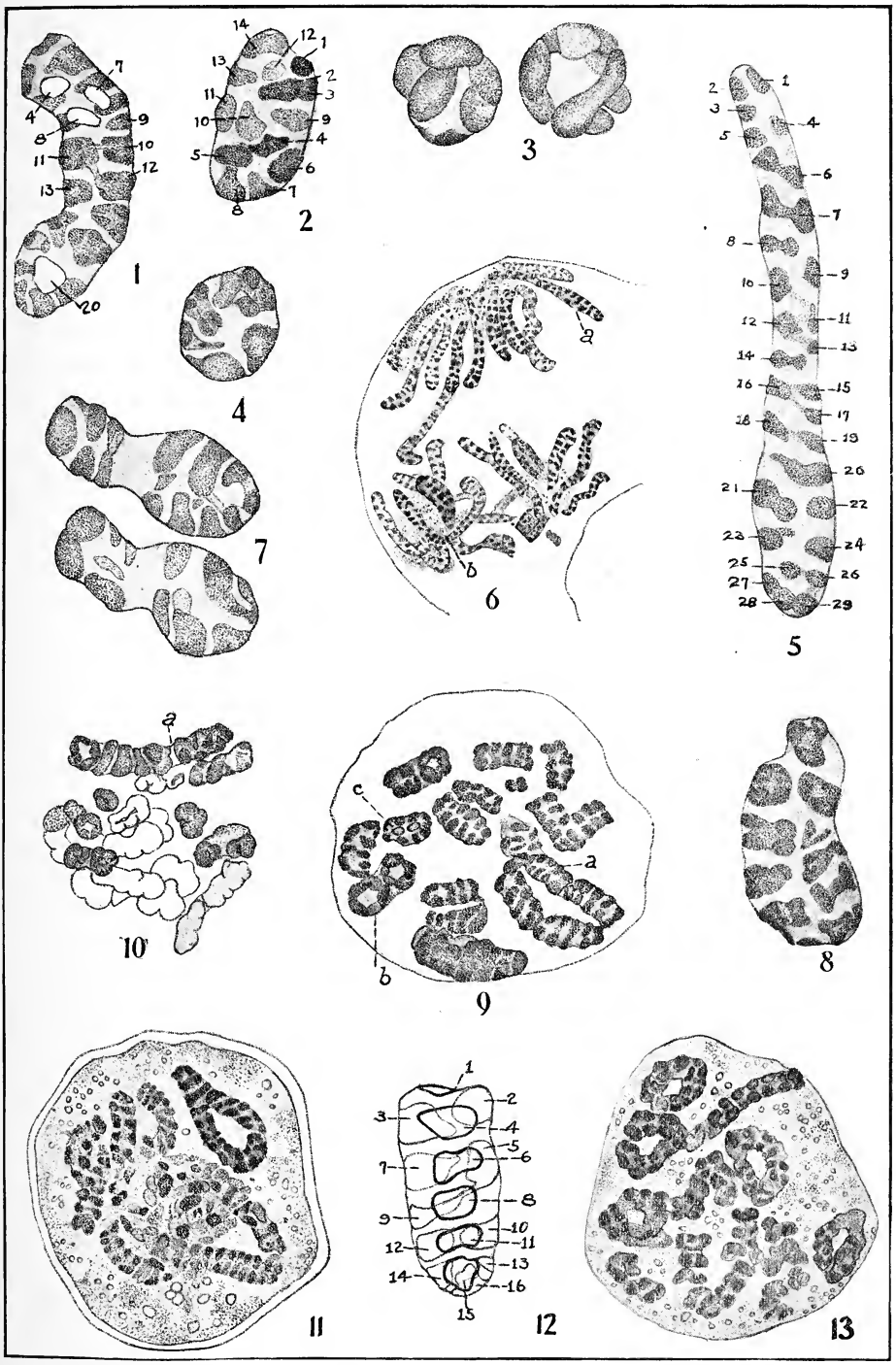
FIG. 15. The same stage in a staminate hair suspended in 3 percent cane sugar. Living chromosomes.

FIG. 16. An eight-hour preparation of metaphase pollen mother cells stained by 1 : 1 aceto-carmine. The chromomeres are especially clear. *Tradescantia virginica.*

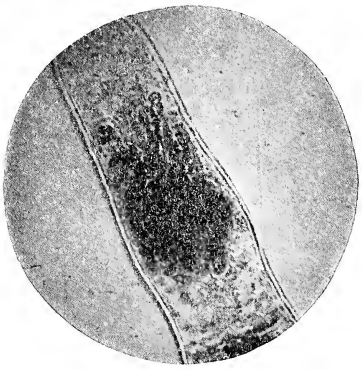
FIG. 17. Similar to figure 16, but showing the hollow construction of the upper chromosome as well as the spiral arrangement of the chromomeres in the linin matrix. *Tradescantia virginica.*

FIG. 18. Metaphase pollen mother cells of Rhoeo suspended in 1 : 1 aceto-carmine. Compare the serial order of the chromomeres with those of figure 17 and figure 16.

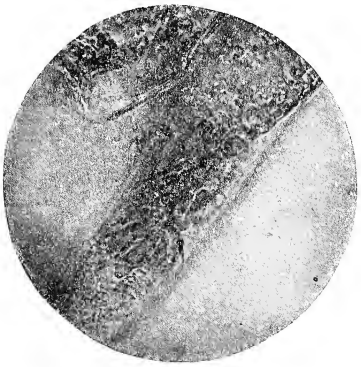
FIG. 19. Anaphase figure in the staminate hair of Tradescantia. Living chromosomes, intended to show the hollow chromosome structure in the two end views in the upper half of the division figure. Suspended in 3 percent cane sugar.



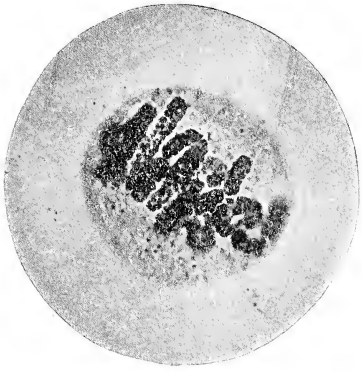
SANDS: STRUCTURE OF CHROMOSOMES



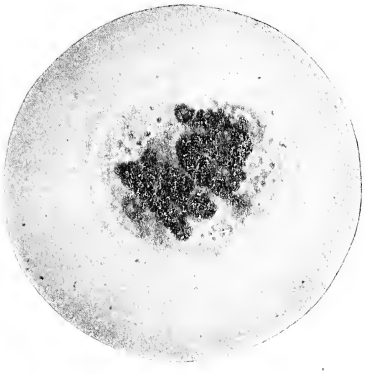
14



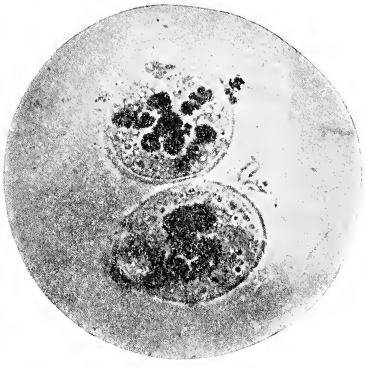
15



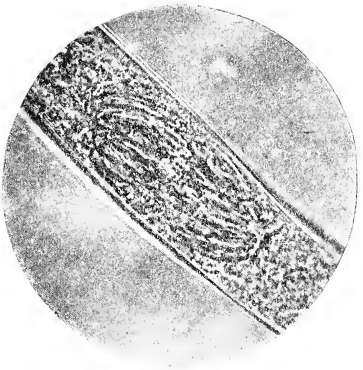
16



17



18



19

SANDS: STRUCTURE OF CHROMOSOMES

THE EFFECT OF RINGING A STEM ON THE UPWARD TRANSFER OF NITROGEN AND ASH CONSTITUENTS

Otis F. Curtis

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From a variety of experiments conducted to determine the effect of ringing on the upward transfer of carbohydrates (Curtis, 1920), evidence was obtained indicating that these foods are carried up the stem chiefly through the phloem tissues even though much of the food to be transported is present as sugar or starch in the xylem tissues. At least the upward transfer of sugars seems to be very clearly checked by the removal of a ring of phloem. When it was realized that sugar may be present in the vessels of the xylem at the time water is being carried in these tissues and yet may not be carried past a region where a ring of phloem is removed, the question immediately presented itself as to whether the commonly accepted idea that nutrients are carried in the "transpiration stream" is a correct one.

Though the literature is full of statements to the effect that inorganic materials absorbed from the soil are carried through the xylem in the "transpiration stream," these for the most part are based on mere assumption or on evidence which is far from conclusive. Certain facts have commonly been offered as proof of rapid transfer in the "transpiration stream." One of the commonest of these is that salts are dissolved in the soil water, and, as the water is known to be carried more or less rapidly through the xylem to the transpiring tissues, at first thought it would seem perfectly logical that the nutrients dissolved in the water would be absorbed and carried with it. If this were true, it would readily account for the rapid absorption and distribution of salts, but since the movement of water and that of solutes through osmotic membranes may be independent of each other, the salts are not necessarily carried with the water. Evidence showing that there is no direct relation between water absorption and salt absorption has been presented in the work of Muenscher (1922) and others. Whether or not the movement of water after absorption influences the transfer of solutes depends in part on whether the solutes get into the water-conducting tissues, and in part on whether these tissues form continuous open tubes with few or no obstructions. It would also depend on the method of water movement, that is, whether the movement is by mass flow throughout, or by diffusion throughout, or, as is perhaps more probable, by mass flow through certain regions and diffusion through others. The possible presence of associated parenchymatous cells, with-

drawing solutes from, or secreting them into, the vessels carrying the water, might also influence their movement.

The fact that solutes have actually been found in the water-conducting vessels and that they have been obtained from cut and bleeding stems has been offered as proof that they are carried with the water. As just stated, however, unless it is known that the water moves by mass flow through open tubes and not by diffusion or through occasional membranes, or unless it is known that neighboring cells do not remove solutes from a passing stream, perhaps transferring them and reintroducing them at a lower level, this can not be considered as conclusive evidence. Furthermore, the method of flow or bleeding from a cut stem may be different from that in a normal uninjured stem in the same sense that a movement of water through a series of cells with membranes enclosing solutions may be very different from the flow that would occur if some of the membranes were cut open.

The fact that, when cut stems are placed in solutions of various dyes, the solutes can be found at considerable distances in the xylem tissues has been considered as proof that solutes must be carried with the water. Normally, however, the water-conducting system may be considered as a closed system with no actual openings. The objections offered to the evidence from cut and bleeding stems should, therefore, be considered in this type of experiment also. Experiments involving the injection of dyes or other solutes through incisions of one type or another might be invalid for the same reason.

A few experiments have been reported in which the movements of dyes or of salts, such as those of lithium or iron, have been studied in rooted and uninjured plants, and some of these indicate a movement, or in some cases an accumulation, in the xylem; but, because of the toxicity of the materials used or of their tendency to be taken up by thick cell walls, or, as with lithium, the ease with which it penetrates membranes and the lack of quantitative determinations, these experiments have not yet given clear indications as to the method of movement or as to the tissues concerned in the movement of nutrient salts.

Since nitrogen is so commonly deficient in soils, and since the absorption of nitrogen can be relatively easily determined both quantitatively by analysis and qualitatively by the color of the plant and by its texture and growth responses, experiments were planned to determine the effects of ringing on the upward movement of nitrogen in stems. In a number of cases the ash contents of the leaves were also determined.

In most of the ringing experiments, twigs or branches were selected in pairs so that the ringed and the normal, unringed branches were similar in size and position. The precaution was also taken to select a branch for ringing such that there were always branches below the ringed one, so that the roots and trunk below would be well supplied with food and their

absorptive ability would not be lost through death or starvation. All ring wounds were covered at the time of ringing with warm paraffin. This precaution was necessary, since otherwise the parts above the ring would often wither. Paraffin instead of vaseline or grafting wax was used, for in previous tests it was found that vaseline was often injurious to stems and that grafting wax was injurious or less easily applied. Immediately after ringing, nitrogen as sodium nitrate was added to the soil. In a few cases calcium nitrate, calcium chloride, and sodium chloride were also used.

In every case the areas of the leaves, as well as the dry weights, were determined. The areas were determined by the use of a planimeter, and readings were determined to tenths of a square inch. With small leaves, like those of Ligustrum, it was found that the error in measurement was greater than that for the larger leaves. In most cases, each lot of leaves of known area was divided into halves or fourths for analysis, one of the halves or two of the fourths commonly being reserved for ash determination. Total nitrogen was determined by the Gunning method as modified to include nitrate nitrogen. The ash was obtained by combustion of the material in an electric furnace at a low red heat.

Since peach trees (*Prunus persica* Sieb. and Zucc.) distinctly show a deficiency of nitrogen by the formation of yellowish leaves and by their slow growth, and since they respond so readily to nitrate applications, experiments were first tried with these. In all the experiments with peach trees, branches one to three centimeters in diameter on young trees were selected. In ringing, bands of bark from one to two centimeters in width were removed.

In the first series, the branches were ringed in May just before the leaves were formed. At the time of collecting the leaves, those from the ringed branches were distinctly smaller; they were thick and stiff, yellowish in color tinged with red, and the younger leaves at the tip of the stem were very much curled. The check leaves from the unringed stem, on the other hand, were larger, thin and pliable, dark green, and not curled. By the end of the season the unringed branches had produced shoots several times as large as those of the ringed branches. For tree number 1, the three largest shoots developing from buds near the apex of the ringed branch averaged only 31 cm. in length. They had no side branches and only 50 leaves, while the three largest shoots on the unringed branch averaged 149 cm. in length. In addition, the check branches produced from 22 to 25 lateral twigs. These secondary twigs of the check were from 8 to 30 cm. long, and the total number of leaves was somewhat over 1,200.

From observations of the color and texture, it would appear as if the ring had checked growth by preventing the upward movement of nitrogen. It is possible, however, that the check in growth and the color of the leaves, as well as their texture, resulted from an excess of carbohydrates in those parts above the ring rather than from a deficiency of nitrogen. The results of analyses for the nitrogen and ash contents of these leaves are presented in table 1.

TABLE 1. *Effect of ringing on the nitrogen content of peach trees when the ring was made just as the buds were starting in the spring. Trees ringed May, 1920, and $NaNO_3$ added to the soil at this time*

Number of Tree	Branch	Date of Sampling	Number of Leaves	Area (sq. dec.)	Dry Weight (g.)	Total Nitrogen (mg.)	Nitrogen per sq. dec.		Nitrogen per g. Dry Wt.		Total Ash (mg.)	Ash per sq. dec.		Ash per g.	
							mg.	Relative to Check	mg.	Relative to Check		mg.	Relative to Check	mg.	Relative to Check
I	Check	7/21	10	3.779	2.0544	88.2	23.3	1.00	42.9	1.00					
I	Ringed	7/21	10	3.389	2.4500	56.0	16.5	.708	22.9	.532					
	Check														
I	*a*	8/27	20	9.257	6.398	246.40	26.7		38.5		388.0	41.9		60.6	
I	*b*	8/27	20	9.900	6.592						436.0	44.0		66.1	
I	*c*	8/27	20	9.404	6.647	245.84	26.1		37.0		397.8	42.3		59.9	
I	*d*	8/27	20	9.578	6.7334	252.56	24.6		37.5		355.0	37.1		52.7	
I	*e*	8/27	20		6.2573	232.89			37.2		319.8			51.1	
I	*f*	8/27	20		6.3074	222.60			35.3		339.6			53.8	
Average							25.8	1.00	37.1	1.00		41.3	1.00	57.3	1.00
	Ringed														
I	*a*	8/27	20	5.495	4.266	96.88	17.6		22.7		169.4	30.8		38.6	
I	*b*	8/27	20	6.011	4.905						180.0	29.8		36.7	
I	*c*	8/27	20	5.663	4.230	100.24	17.7		21.2		193.4	34.2		40.9	
I	*d*	8/27	20	5.521	4.6646	99.40	18.0		21.3		171.2	31.0		36.6	
I	*e*	8/27	20		4.7188	101.36			21.5		166.0			35.2	
I	*f*	8/27	20		4.2273	100.80			23.8		176.6			41.8	
Average							17.7	.689	22.1	.596		31.5	.761	38.3	.668

TABLE 1. *Continued.*

Number of Tree	Branch	Date of Sampling	Number of Leaves	Area (sq. dec.)	Dry Weight (g.)	Total Nitrogen (mg.)	Nitrogen per sq dec.		Nitrogen per g. Dry Wt.		Total Ash (mg.)	Ash per sq. dec.		Ash per g.	
							mg.	Relative to Check	mg.	Relative to Check		mg.	Relative to Check	mg.	Relative to Check
2	Check	7/19	10	4.547	2.8461	113.12	24.9	1.00	39.7	1.00					
2	Ringed	7/19	10	2.831	1.8749	46.62	16.5	.662	24.9	.626					
2	Check	7/21	10	4.063	2.357	95.90	23.6	1.00	40.7	1.00					
2	Ringed	7/21	10	2.728	1.8094	43.82	16.1	.680	24.2	.595					
	Check														
3	*a*	8/12	10	5.031	3.2160	122.36	24.3		38.0		239.8	47.6		74.5	
3	*b*	8/12	10	4.515	2.8216	97.44	21.6		34.5		180.2	39.9		63.8	
Average							22.9	1.00	36.2	1.00		43.8	1.00	69.1	1.00
	Ringed														
3	*a*	8/12	10	3.909	2.9540	65.62	16.8		22.2		*151.0	*38.8		51.1	
3	*b*	8/12	10	4.147	3.3693	68.6	16.5		20.4		123.8	29.8		36.7	
Average							16.6	.726	21.3	.586		34.3	.785	43.9	.499
	Check														
4	*a*	8/12	10	4.889	2.9634	107.24	21.9		36.2		165.2	33.8		55.7	
4	*b*	8/12	10	5.063	3.1846	110.04	21.7		34.5		221.2	43.6		69.4	
Average							21.8	1.00	35.3	1.00		38.7	1.00	62.6	1.00
	Ringed														
4	*a*	8/12	10	3.831	3.0043	71.12	18.6		23.7		*140.2	*36.6		*46.3	
4	*b*	8/12	10	3.515	2.8295	60.76	17.3		21.5		*125.0	*35.5		*44.1	
Average							17.9	.821	22.6	.638		36.1	.932	45.2	.720

* When the ash was taken out of these crucibles, it was found that some carbon still remained. This would explain the high readings.

It is evident from the data on the nitrogen content that ringing has resulted in a lessened amount of nitrogen in the leaves whether expressed as milligrams of nitrogen per leaf, per gram of dry matter, or per unit of leaf area. The small amount of nitrogen per leaf and per unit of weight might be expected, because the leaves are smaller and the dry weights are relatively high because removal of the carbohydrate has been retarded by the ring. The small amount of nitrogen per unit of leaf area, however, is a good indication that relatively less had been carried up the ringed stem, for otherwise one would expect more nitrogen as the leaves are thicker and heavier per unit of area. Furthermore, there was nothing to prevent the removal of proteins or other forms of nitrogen from the check leaves back to the trunk or roots, while such removal from the other leaves would have been retarded by the ring. The total amount of nitrogen moving up the unringed stem was certainly much greater than that moving up the ringed stem, for there were from ten to twenty times as many leaves on the check stem, and these leaves were also larger, usually from 30 to 70 percent, than those of the ringed stem.

Though nitrates have been found in abundance in stems (Berthelot and André, 1886), and organic nitrogen compounds have been found in solutions bleeding from cut stems (Schroeder, 1871), it is conceivable that much of the nitrogen might be transformed into protein which probably would be more readily carried in the phloem. In order, therefore, to determine if the rings had also retarded the upward movement of those nutrients which might be less likely to combine with organic matter and which have been found in abundance in the solutions bleeding from cut stems (Schroeder, 1871, and others), and which, therefore, are commonly considered to be carried in the "transpiration stream," the ash contents of a number of samples were determined. These data are also presented in table 1 for comparison with the nitrogen analyses.

In the most complete and dependable set of analyses, those for tree number 1, the leaves from the ringed stem have only 76 percent as much ash per unit of area as the checks, while per unit of dry weight they have only about 67 percent as much. The few analyses of samples from the other trees indicate similar differences.

Another experiment in some respects comparable with this one with peach trees was tried with a lilac bush. A stem three centimeters in diameter was ringed August 6, 1920. No leaf samples were taken at this time, however, and no nitrate was added to the soil. On July 30 of the following year (1921) other stems of this same bush were ringed and sodium nitrate was added to the soil. Leaf samples from the branch ringed the previous year, as well as from the newly ringed and check branches were taken at this time and again on August 22 and September 9. The shoot growth on the ringed stem was distinctly less than that on the check stem, the leaves were smaller, thicker, less pliable, and were distinctly yellowish

TABLE 2. *Effect of ringing on the nitrogen content of lilac leaves (Syringa vulgaris) when the ring is made the previous season. Stem ringed August 6, 1920. $NaNO_3$ added to soil July 30, 1921*

	Date of Sampling	Number of Leaves	Area (sq. dec.)	Dry Weight	Nitrogen Total (mg.)	Nitrogen per sq. dec.		Nitrogen per g.		Ash Total mg.	Ash per sq. dec.		Ash per g.	
						mg.	Relative to Check	mg.	Relative to Check		mg.	Relative to Check	mg.	Relative to Check
Check *a*	7/30	10	2.812	3.9463	97.72	34.8		24.8		282.8	100.5		71.7	
b	7/30	10	2.509	3.3832	85.40	34.0		25.2		248.2	98.9		73.3	
Average			2.711	3.6648	91.56	34.4	1.00	25.0	1.00	265.5	99.7	1.00	72.5	1.00
Ringed *a*	7/30	11	1.871	3.1778	43.96	23.5		13.8		111.0	59.3		34.9	
b	7/30	11	1.632	2.8111	40.32	24.7		14.3		96.2	58.9		34.2	
Average*			1.592	2.7222	42.14	24.1	.701	14.0	.560	94.2	59.1	.593	34.6	.477
Check *a*	8/22	10	2.864	4.148	112.56	39.3		27.1		382.8	133.6		92.2	
b	8/22	10	2.903	3.824	98.84	34.0		25.8		326.8	112.5		85.4	
Average			2.884	3.986	105.70	36.7	1.00	26.5	1.00	358.8	123.1	1.00	88.8	1.00
Ringed *a*	8/22	13	2.335	4.435	53.76	23.0		12.1		147.2	63.0		33.1	
b	8/22	13	2.780	4.787	59.60	21.4		12.5		160.8	57.8		33.5	
Average*			1.968	3.547	43.60	22.2	.606	12.3	.464	118.5	60.4	.491	33.3	.375
Check *a*	9/9	12	3.347	4.4344	112.84	33.7		25.4		352.0	105.1		79.3	
b	9/9	12	3.457	4.8689	127.40	36.9		26.2		380.6	110.0		78.1	
c	9/9	12	3.257	4.6588	123.78	38.0		26.6		342.8	105.2		73.5	
d	9/9	12	3.425	4.8406	129.36	37.9		26.7		350.4	102.3		72.3	
Average*			2.810	3.9173	103.20	36.6	1.00	26.2	1.00	297.0	105.7	1.00	75.8	1.00
Ringed *a*	9/9	16	2.515	4.4514	52.08	20.7		11.7		165.4	65.7		37.1	
b	9/9	16	2.457	4.3193	50.68	20.6		11.7		159.6	64.9		36.9	
c	9/9	16	2.670	4.7939	57.12	21.4		11.9		183.0	68.5		38.1	
d	9/9	16	2.496	4.9253	55.44	22.3		11.3		178.4	71.4		36.2	
Average*			1.584	2.8890	33.64	21.2	.580	11.7	.444	107.3	67.6	.640	37.1	.489

* Averages for ten leaves.

green in color with an abundance of red pigment. Analyses of the leaves from the branch that had been ringed the previous year and from the check branches are presented in table 2.

The data show that at the time of adding the nitrate (July 30), nearly a year after ringing, the check leaves had about 43 percent more nitrogen per unit of area than had the leaves of the ringed stem, that by August 22 they had 65 percent more, and by September 9, 73 percent more. On July 30 the check leaves had 1.79 times as much nitrogen per unit of dry weight as the ringed leaves, on August 22, 2.12 times, and on September 9, 2.24 times.

The ash analyses clearly indicate that the ring has hindered the movement of ash constituents also, the check leaves on July 30, August 22, and September 9 having respectively ash contents per unit of area of 1.64, 2.04, and 1.56 times that of the leaves from the ringed stem.

From these experiments with peach and lilac, it is very evident that the ringing has hindered the upward movement through the stems of both nitrogen and ash constituents. One could not conclude from these experiments alone, however, that the nitrogen or ash constituents move chiefly through the phloem instead of the xylem, because it might be true that they move up very largely through the newly formed xylem cells. The ring has not only cut the phloem, but has also prevented the formation of new xylem at the point of ringing. Furthermore, the changed growth above the ring might in some other way influence the nitrogen movement.

In order to eliminate some of these difficulties, another set of experiments was started after the shoots had become well developed and the leaves were mostly formed. Samples of leaves were taken at the time the rings were made and nitrate spread on the soil, and again later at different intervals. Because of an accident to the drying oven, most of the samples taken on the day of ringing were lost, and a second set was taken two days later but many of these also were later lost. These trees were growing in a poor, sandy soil, and at the beginning of the experiment the leaves were yellowish green and very characteristic of peach trees grown in soils deficient in nitrogen. A few weeks after applying the nitrate, the leaves on all excepting the ringed branches became darker green, and in many cases there seemed to be a renewal of shoot growth. The results of the analyses of this set are presented in table 3. For tree *C*, all the samples taken at the time of ringing were lost except two, one of which consisted of the younger leaves from near the tips of several branches and the other of older, basal leaves. When samples were taken from this tree on October 7, the upper leaves were kept separate from the basal leaves. The young upper leaves from the ringed stem were very deficient in chlorophyll and were much curled.

It is evident from these results that, even after the new xylem and leaves have been developed, the cutting of the phloem has hindered the

movement of nitrogen into the leaves. The small number of samples collected at the beginning of the experiment has not allowed for any very accurate determinations of the percentages of increase. Yet the evidence is fairly clear that the ring has not entirely prevented the upward transfer of nitrogen, for the leaves above a ring show an increase per unit of area, but this increase is very much less than that in the check leaves. It is interesting to note that in the one instance where a narrow strip of phloem had grown over the ring, thus bridging the gap, the increase in nitrogen

TABLE 3. *Effect of ringing on the nitrogen content of peach leaves when the ring is made after the leaves have formed and the shoot growth is practically completed*

Tree	Branch	Treatment	Date Sampled	Number of Leaves	Area (sq. dec.)	Dry Wt. (g.)	Nitrogen Total (mg.)	Nitrogen per sq. dec.		Increase per Unit Area %	Nitrogen per g.	
								mg.	Rel.		mg.	Rel.
A	1′	Check	7/21	10	3.921	2.888	71.18	18.2	100		24.6	100
A	1	Ringed	7/19	10	4.469	3.330	84.42	18.9			25.4	
A	1	Ringed	7/21	10	4.592	2.993	80.64	17.6			26.9	
A	2	Ringed	7/21	9	3.921	2.433	66.78	17.0			27.4	
		Average						17.8	98		26.6	101
A	1′	Check (*a*)	8/12	10	4.431	3.207	116.76	26.4			36.4	
		(*b*)	8/12	10	4.560	3.156	114.66	25.1			36.4	
		Average						25.8	100	42%	36.4	100
A	1	*Ringed (*a*)	8/12	10	4.431	3.765	112.16	25.3			29.8	
		(*b*)	8/12	10	4.669	3.889	115.08	24.6			29.6	
		Average						*25.0	*97	*40%	29.7	*82
A	2	Ringed (*a*)	8/12	10	4.082	3.746	82.88	19.3			21.2	
		(*b*)	8/12	10	4.302	3.906	82.88	20.3			22.1	
		Average						19.8	77	11%	21.7	60
A	2′	Check (*a*)	8/27	20	9.011	6.757	246.12	27.3			36.4	
		(*b*)	8/27	20	8.753	6.720	241.36	27.6			35.9	
		Average						27.5	100	51%	36.2	100
A	2	Ringed (*a*)	8/27	20	7.637	8.591	164.08	21.5			19.1	
		(*b*)	8/27	20	7.982	8.435	159.60	19.9			18.9	
		Average						20.7	75	16%	19.0	53
B	1′	Check	7/21	10	4.696	3.065	96.18	20.5			31.4	
	2′	Check	7/21	10	4.460	2.836	90.30	20.2			31.8	
		Average						20.4	100		31.6	100
B	1	Ringed	7/21	10	4.651	2.875	81.48	17.5			28.3	
	3	Ringed	7/21	10	3.753	2.393	63.00	16.7			26.3	
		Average						17.1	84		27.3	86
B	3′	Check (*a*)	10/7	22	9.714	6.939	280.56	29.4			40.8	
		(*b*)	10/7	22	9.391	7.270	286.44	30.5			39.4	
		Average						29.9	100	46%	40.1	100
B	3	Ringed (*a*)	10/7	22	7.005	6.465	131.04	18.7			20.3	
		(*b*)	10/7	22	7.011	6.169	127.68	18.2			20.7	
		Average						18.5	62	8%	20.5	51

* A narrow strip of tissue had developed bridging the ring on this branch.

TABLE 3. *Continued.*

Tree	Branch	Treatment	Date Sampled	Number of Leaves	Area (sq. dec.)	Dry Wt. (g.)	Nitrogen Total (mg.)	Nitrogen per sq. dec.		Increase per Unit Area %	Nitrogen per g.	
								mg.	Rel.		mg.	Rel.
C		Leaves close to tip	7/21	10	3.477	2.010	73.78	21.2			36.7	
		Leaves close to base	7/21	10	5.328	3.140	100.38	18.8			32.0	
C_2		Upper leaves										
		Check (*a*)	10/7	10	4.199	3.5679	128.52	30.6			36.0	
C_2		(*b*)	10/7	10	3.857	3.2891	118.72	30.8			36.1	
C_2		(*c*)	10/7	10	3.670	3.0776	112.84	30.7			36.5	
C_2		(*d*)	10/7	10	4.076	3.3948	123.90	30.4			36.5	
C_2		(*e*)	10/7	10	4.283	3.7979	133.28	31.1			35.1	
		Average						30.7	100		36.0	100
		Upper leaves										
C_2		Ringed (*a*)	10/7	10	2.706	2.1400	30.24	13.7			14.1	
C_2		(*b*)	10/7	10	2.129	2.0087	27.58	13.0			13.7	
C_2		(*c*)	10/7	10	1.870	1.6834	23.94	12.8			14.2	
C_2		†(*d*)	10/7	10	4.644	4.9979	81.34	†17.5			†16.3	
		Average						13.2	43		14.0	39
		Lower leaves										
C_2'		Check (*a*)	10/7	10	5.263	3.623	115.92	22.0			32.0	
C_2'		(*b*)	10/7	10	5.412	3.9376	130.76	24.2			33.2	
C_2		(*c*)	10/7	10	5.198	3.6550	101.08	19.4			27.7	
C_2		(*d*)	10/7	10	4.599	3.1287	96.04	20.9			30.7	
C_2		(*e*)	10/7	10	4.934	3.7445	133.00	27.0			35.5	
		Average						22.7	100		31.8	100
C_2		Ringed (*a*)	10/7	10	4.702	4.1740	75.64	16.1			18.1	
C_2		(*b*)	10/7	10	4.212	3.5381	62.44	14.8			17.7	
C_2		(*c*)	10/7	10	4.270	3.7556	64.12	15.0			17.1	
C_2		(*d*)	10/7	10	4.425	3.9574	70.56	15.9			17.8	
C_2		(*e*)	10/7	10	3.960	3.3688	61.04	15.4			18.1	
		Average						15.5	68		17.8	56

† These leaves were rolled like the others but were larger and more basal; not included in average.

above the ring was practically the same as that in the check. This ability of a narrow strip of phloem to allow for the transfer of an approximately normal quantity of nutrients is comparable to its ability to allow sufficient foods and nutrients to pass to insure approximately normal growth, as was previously found (Curtis, 1920).

A somewhat similar experiment was performed with a lilac bush (*Syringa vulgaris* L.). In this case paired twigs were selected, and one of each pair was ringed just above the fork. The rings were made on the current year's growth. It was hoped that it would be possible to analyze each twig

TABLE 4. *Syringa vulgaris. Effect of ringing on the upward transfer of nitrogen and ash constituents when the ring is made after the leaves are formed and shoot growth is completed. Ringed July 30, 1921*

	Date of Sampling	Number of Leaves	Area (sq. dec.)	Dry Weight	Nitrogen Total (mg.)	Nitrogen per sq. dec.		Nitrogen per g.		Ash Total (mg.)	Ash per sq. dec.		Ash per g.	
						mg.	Relative to Check	mg.	Relative to Check		mg.	Relative to Check	mg.	Relative to Check
Check *a*	7/30	10	3.335	4.5008	114.80	34.4		25.5		322.6	96.7		71.6	
b	7/30	10	3.315	4.1064	106.96	32.3		26.0		293.8	88.6		71.5	
c	7/30	11	3.586	4.6192	115.92	32.3		25.1		330.6	92.2		71.6	
Average*			3.303	4.2688	109.05	33.0	1.000	25.6	1.000	305.6	92.5	1.000	71.6	1.000
Ringed *a*	7/30	10	3.354	4.4176	115.36	34.4		26.1		307.6	91.7		69.6	
b	7/30	10	3.360	4.2288	110.32	32.8		26.1		320.2	95.3		75.7	
c	7/30	11	3.586	4.6338	117.96	32.9		25.5		332.6	92.7		71.8	
Average*			3.325	4.3196	110.97	33.4	1.011	25.9	1.013	310.1	93.2	1.008	72.4	1.011
Check *a*	8/22	10	3.322	4.6478	127.12	38.3		27.4		357.2	107.5		76.8	
b	8/22	10	3.264	4.6019	124.80	38.2		27.1		358.0	109.6		77.8	
c	8/22	10	3.231	4.5624	117.04	36.3		25.7		318.8	98.6		69.8	
Average			3.272	3.4530	122.99	37.6	1.000	26.7	1.000	344.7	105.2	1.000	74.8	1.000
Ringed *a*	8/22	10	3.709	6.0101	102.48	27.6		17.0		315.8	85.1		52.5	
b	8/22	10	3.496	6.4031	109.76	31.4		17.1		351.6	100.5		54.9	
c	8/22	10	3.670	5.8524	106.40	29.0		18.2		322.8	87.9		55.1	
Average			3.625	6.0885	106.21	29.3	0.771	17.5	0.653	330.1	89.2	0.848	54.2	0.725

* Averages for 10 leaves.

separately, but there were so few leaves on a twig and these were so variable in size that thirty leaves were taken from each treatment and these were divided into three samples each for analysis. The day after the nitrate was added heavy rains washed it into the soil. The analyses are presented in table 4.

The data indicate that the leaves from the check stem increased in nitrogen content per unit of area, while those from the ringed stem have slightly decreased. A similar gain by the check leaves and loss from the ringed is apparent in the ash analyses.

Similar experiments were performed in 1920 with *Acer platanoides* L., *Prunus pennsylvanica* L., and *Pyrus communis* L. In these cases the experiments were started in August, and there were no heavy rains to wash the nitrate into the soil until some time after it was added. The analyses of Prunus indicated a small increase of nitrogen in the check leaves and a still smaller increase in the leaves from the ringed stem, but the gain in the check was so small, only about 6 percent per unit of area, that the data are not presented. When expressed as percentage of dry weight, there was a distinct lowering in the leaves of the ringed stem, as would be expected. The analyses of the maple leaves picked 56 days after the nitrate was added to the soil showed no increase in nitrogen even in the check leaves. It would seem that all nitrogen absorbed had been taken up by other tissues before it could reach the leaves. This might be expected at this time of year, since quantities of carbohydrates must have accumulated in the various storage tissues. It is interesting to note that, though there was no change in nitrogen as measured per unit of area, the leaves from the ringed stems developed a very distinct purplish color, whereas the check leaves remained green. When the nitrogen content was expressed as percentage of dry weight, there was a distinct lowering in the leaves of the ringed stems.

From the data thus far presented, it seems evident that the ringing has reduced the upward transfer of nitrogen and ash constituents. It is conceivable, however, that the low content of nutrients in the leaves may be due, at least in part, to the retention of these constituents in the stem just above the ring, since these tissues have an excess of organic matter, the removal of which is prevented by the ring.

In order to test this point, an experiment was tried with California privet (*Ligustrum ovalifolium* Hassk.) in which pairs of uniform stems were used and both the leaves and the entire experimental stem were analyzed. In preliminary experiments it was found that the areas, weights, and nitrogen and ash contents of paired leaves of a given stem were very nearly equal. One from each pair of the more mature leaves of a given stem were removed at the beginning of the experiment, and the remaining leaves, together with the stems, were taken at the close. Three to six pairs of the younger leaves growing at the tops of the stems were left and

TABLE 5. *Effect of ringing on the nitrogen and ash contents of leaves and stems of Ligustrum. All stems ringed August 25 and harvested as indicated*

	Averages of 6 Stems						Averages of 6 Stems except as Indicated in Brackets						Averages of 7 Stems except as Indicated in Brackets					
	Check			Ringed			Check			Ringed			Check			Ringed		
Date of Sampling ...	8/25	10/3	*Ave. Gain %	8/25	10/3	*Ave. Gain %	8/25	10/4	*Ave. Gain %	8/25	10/4	*Ave. Gain %	8/25	11/19	*Ave. Gain %	8/25	11/19	*Ave. Gain %
Area of leaves sq. dec.).....	1.037	1.214	18.1	0.955	1.077	14.2	0.874	0.993	15.1	0.848	0.956	11.6	0.996	(6) 1.145	(6) 15.1	0.958	(6) 1.182	(6) 21.9
Dry wt. (g.).....	1.2323	1.5168	25.2	1.1096	1.7115	56.7	1.0272	1.2356	20.6	0.9567	1.6805	75.6	1.0623	1.5164	45.1	1.0652	1.9869	8.3
Nitrogen (mg.)..	17.69	40.41	139.0	15.59	18.99	26.2	15.17	28.61	92.8	(5) 12.6	15.77	19.1	(6) 16.43	40.11	(6) 171.3	(4) 15.40	13.57	(4) 4.8
Nitrogen per sq. dec. (mg.).....	16.58	38.32	100.6	15.82	17.27	10.3	17.55	29.36	64.2	(5) 16.45	16.38	2.6	(6) 16.25	36.40	(5) 139.4	(4) 16.30	(6) 13.21	(4) 9.4
Nitrogen per g. (mg.).....	14.10	26.37	86.4	13.82	10.87	−20.6	14.72	23.24	48.7	(5) 13.97	9.38	−30.4	(6) 17.70	26.45	(6) 83.4	(4) 11.18	7.20	(4) −47.1
Ash (mg.).....	93.5	164.3	73.4	82.4	98.7	23.4	77.3	115.4	48.7	77.1	91.6	18.0	76.3	(5) 126.4	(5) 97.6	76.1	(5) 85.0	(5) 16.4
Ash per sq. dec. (mg.).....	92.7	136.6	47.8	88.3	94.1	6.8	91.3	117.7	29.7	95.5	96.0	0.8	80.8	(5) 124.7	(4) 66.5	77.0	(4) 82.6	(4) 6.1
Ash per g. (mg.).	79.1	108.7	38.4	77.9	59.4	−21.4	76.5	94.1	23.2	81.7	54.8	−32.9	72.3	92.2	32.3	72.7	(5) 45.2	(5) −39.3
Dry weight upper leaves ..														0.8846			0.9159	
Nitrogen per g. upper leaves (mg.).....														25.04			8.58	
Ash per g. upper leaves (mg.)...														75.7			36.3	
Dry wt. stem, basal 20 cm. ..		2.0804			2.1933			1.7960			1.8697			1.9853			1.9059	
Dry wt. stem, upper part....		1.6031			1.7674			1.5154			1.4410			2.1628			2.3123	
Nitrogen per g. stem, basal 20 cm.....		7.518			4.025			5.14			3.54			9.27			6.69	
Nitrogen per g. stem, upper part.....		8.673			3.932			6.65			3.52			10.40			(5) 6.87	

* These are averages of the gains for each stem and not the gain of the averages.

analyzed separately at the close of the experiment. Forty-two stems were selected for the experiment. These were chosen in pairs as they grew on the bushes of a hedge, and one of each pair was ringed while the other was left as a check. Sodium nitrate together with smaller amounts of calcium chloride, sodium chloride, and calcium carbonate were added to the soil around the hedge. The application was not very uniform because the bushes were growing close together and the bunches of sod beneath the bushes made uniform application difficult. This probably accounts very largely for the difference between the series taken October 3 and that taken October 4, since the two series were from different parts of the hedge. The data are presented in table 5.

From these data it is evident that there was a distinctly smaller increase of nitrogen in the leaves of the ringed stems whether this is considered as an increase in absolute quantity of nitrogen, or in the amount per unit of leaf surface. The amount of nitrogen per unit of dry weight shows, in every case, a loss in the leaves of the ringed stems. This was due very largely, of course, to the marked increase in dry weight of those leaves.

The data for the stems show that there is a greater amount of nitrogen per gram of twig in the checks than in the ringed stems. This is true in both the basal and the upper parts of the stem. The smaller amounts in the ringed stems are not merely apparent and due to the greater dry weights of the ringed stems which resulted from an accumulation of food, for, in each of the nineteen pairs of twigs analyzed in the three series, the check stem had not only a greater amount of nitrogen per unit of weight, but it had, with but two exceptions, a greater absolute amount of nitrogen than had the corresponding ringed stem. In one of these cases the basal part of the check stem had an unusually low amount of nitrogen, so low that it was evidently due to an error in analysis, and in the other case the ringed stem was not well matched with the check, but was distinctly larger, which fact would easily account for the greater total nitrogen.

In the series harvested Nov. 19, the wood and bark of the twigs were analyzed separately. The results of these determinations are given in table 6. The data for the leaves and for the entire twigs are included in table 5.

It is evident from table 6 that the ringing has resulted in a distinct increase in the weight of the bark, especially in the twenty centimeters immediately above the ring. This effect on the bark is even more clearly indicated by the increase in the ratio bark: wood, both in the basal and in the upper parts of the stem. The ratio between the amount of nitrogen in the bark and that in the wood is also increased by ringing.

The ash contents of the stems were not determined because of the small amount of material and the difficulty of dividing the samples, but those of the leaves were obtained and are presented in table 5 for comparison with the nitrogen. The results of the ash determinations correspond

very closely to those of the nitrogen determinations. There was a smaller absolute increase in the ash of the leaves from the ringed stems as well as a smaller increase per unit of area and a distinct decrease per unit of weight.

TABLE 6. *The effect of ringing on the nitrogen content of the wood and bark of stems. Averages of seven determinations except where indicated in brackets*

	Check Basal 20 cm.		Ringed Basal 20 cm.		Ave. Ratio Ringed / Check	Check Upper Part		Ringed Upper Part		Ave. Ratio Ringed / Check
		Ave. Ratio Bark / Wood		Ave. Ratio Bark / Wood			Ave. Ratio Bark / Wood		Ave. Ratio Bark / Wood	
Ave. dry wt. wood (g.)	1.4366		1.2217		0.827	(6) 1.3619		(5) 1.3465		(5) 0.943
Ave. dry wt. bark (g.)	0.5467	0.391	0.6842	0.606	1.240	(6) 0.8009	(6) 0.647	(5) 0.9658	(5) 0.819	(5) 1.241
Ave. total nitrogen, wood (mg.)	10.16		5.16		0.462	(6) 10.34		(5) 5.85		(5) 0.457
Ave. total nitrogen, bark (mg.)	8.34	0.854	8.05	1.904	0.915	(6) 12.37	(6) 1.387	(4) 12.58	(4) 2.669	(4) 0.901
Ave. nitrogen per g., wood (mg.)	7.13		3.90		0.545	(6) 7.33		(5) 3.38		(5) 0.499
Ave. nitrogen per g., bark (mg.)	16.11	2.25	11.41	3.15	0.738	(6) 15.43	(6) 2.17	(4) 11.09	(4) 3.14	(4) 0.747
Ave. nitrogen per g. stem (mg.)	9.27		6.69		0.731	10.40		(5) 6.87		(5) 0.682

The data thus far presented clearly indicate that ringing commonly hinders the movement of nitrogen and ash constituents into the tissues above the ring. This cannot be considered as conclusive evidence, however, that the phloem rather than the xylem is chiefly concerned in the movement of these nutrients. I have obtained considerable evidence showing that transpiration from the leaves of the ringed stems is distinctly less than that from normal leaves. This is due, in part at least, to the high concentration of solutes above the ring with a corresponding lowering of vapor pressure, and perhaps also to increased retention by colloids or to morphological or other changes. If the nutrients are carried in the so-called "transpiration stream," and if their rate of movement is determined by the water movement, a check in the transpiration from tissues above a ring would very probably reduce the movement of the solutes into these tissues. In order to eliminate this factor of transpiration, twigs of privet were experimented with as described below.

Twelve sets of three stems each were selected for the experiment. In every twig the young growing shoot was removed and four pairs of leaves were left at the apex. The four pairs of leaves immediately below these

were removed, leaving the twig bare as indicated in figure 1. On one twig from each set (no. 1) a ring was made immediately below the upper leaves. On a second twig (no. 2) in each set two rings were made, one just below the upper leaves and a second at the base of the defoliated part. The third twig (no. 3) was ringed at the base of the defoliated part and not at the point just below the upper leaves. Sodium nitrate was added to the soil around

FIG. 1. Showing method of ringing so that the amount of water passing through the defoliated part of each stem would be approximately the same whether or not this part is isolated from the roots by a ring. 1. Check, medium carbyhodrate content in defoliated part. 2. Ringed, low carbohydrate content in defoliated part. 3. Ringed, high carbohydrate content in defoliated part.

the bushes at the time of ringing, August 25, and the twigs were cut for analysis on October 3. An analysis was made of the defoliated part of each stem as indicated by brackets in the figure. It was assumed that the amount of water moving through the stem would be approximately the same in numbers 1 and 2, since both had the same number of leaves on the stem at the apex and both were ringed immediately below these leaves. Since the defoliated part of the stem in no. 1 would probably have a higher sugar content than number 2, as it was not isolated by a ring from the leaves at the base, and since any such difference in carbohydrate content might distinctly alter its tendency to retain nitrogen, the third twig

TABLE 7. *Effect of ringing on the nitrogen contents of twigs when transpiration from ringed and check stems is approximately equal and where the carbohydrate content is low in one treatment and high in another. The ratios are averages of the ratios for each set, not ratios of averages**

Treatment	No.	Wood										Bark						Entire Stem	
		Dry Weight		Total Nitrogen		Nitrogen per g.		Volume		Nitrogen per cc.		Dry Weight		Total Nitrogen		Nitrogen per g.		Nitrogen per g.	
		g.	Rel. to Check	mg.	Rel. to Check	mg.	Rel. to Check	cc.	Rel. to Check	mg.	Rel. to Check	g.	Rel. to Check	mg.	Rel. to Check	mg.	Rel. to Check	mg.	Rel. to Check
Check.....	1	.7858	1.00	5.20	1.00	6.730	1.00	10.616	1.00	.5326	1.00	†(.4795) .4586	1.000	†(6.91) 6.63	1.00	†(14.492) 14.053	1.00	†(9.621) 9.497	1.00
Ringed, low carbohydrate	2	.6077	0.769	2.70	0.543	4.672	0.698	9.100	0.884	.3011	0.610	.3808	0.828	4.75	0.745 †(1.017)	12.533	0.889 †(0.728)	7.562	0.822 †(0.599)
Ringed, high carbohydrate	3	1.0797	1.616	3.99	0.723	3.219	0.419	12.938	1.328	.1280	0.455	.6242	1.497	6.76	1.135	10.778	0.776	6.078	0.633

* For no. 1 these are averages of 12 stems, except for volumes, which are averages of 11; no. 2, averages of 10 stems; no. 3, averages of 9 stems, except for volumes, which are averages of 8 stems.

† Including the ringed twig, which had healed over, in place of its check which showed an excessively low nitrogen content, evidently an error in analysis.

in each set was ringed only at the base of the defoliated part so as to allow the carbohydrate to move down from the upper leaves. Though no measurements were made, it was assumed that transpiration from these stems would approximate that from stems 1 and 2.

When the stems were cut they were tested with iodin to ascertain any evident differences in their starch contents. In the check (no. 1) starch was abundant in the xylem. In no. 2 no trace of starch could be detected, and in no. 3 starch was evidently somewhat more abundant than in no. 1. Averages of the data obtained are presented in table 7.

As indicated by the table, the bark and wood were analyzed separately. The volume of each twig was calculated from the length and the diameters at each end. Analyses of the wood showed that the ringing at the base of the defoliated part had resulted in a lower nitrogen content of that part whether measured as total nitrogen per stem, as nitrogen per gram of dry weight, or as nitrogen per unit of volume. This was true in each of the twelve sets with but two exceptions. One of the exceptions, in fact, adds weight to the evidence, for in this one twig the ring had healed over. The data for this one twig are not included in the averages. In the other exception, the difference was slight and probably due to inaccurately paired twigs. It should be mentioned in this connection that these twigs showed much greater individual variations than did the leaves. The nitrogen contents of the xylem, for example, expressed as percentages of dry weight, varied in the check twigs from a minimum of 4.21 percent to a maximum of 9.95 percent. For each set of three twigs, however, care was taken to select those similar in position, size, and color of leaves, and, with the exception just mentioned, the check in each set of three always had the higher nitrogen content.

The data also show that the effect of ringing on the nitrogen content of the wood above the ring is independent of its effect on the carbohydrate content. In one treatment (no. 2) the carbohydrate content was much reduced by the ring, as evidenced by the tests for starch and by the dry weights. In the other treatment (no. 3) it has increased the carbohydrate content as indicated by the same tests, yet in both cases the nitrogen content is distinctly reduced. In no. 2, the dry weight of the wood was reduced on the average by about 23 percent, the total nitrogen 46 percent, and the nitrogen per gram 30 percent. In no. 3, the dry weight was increased 62 percent, the total nitrogen was reduced 28 percent, and the nitrogen per gram was reduced 58 percent.

Analyses of the bark show that the rings at the bases of the defoliated stems resulted in a distinct reduction of the nitrogen in those which had a low carbohydrate content, but had practically no influence on those which had excess carbohydrate. The amount of nitrogen per gram of material seems to be clearly reduced. In two instances, not including the stem which had healed, there was a slightly higher amount of nitrogen

per gram of bark in the ringed stems with low carbohydrate (no. 2). This is probably in part accounted for by the distinctly lower dry weight of the bark of these stems, as there was no phloem connection with a carbohydrate supply. In no. 2, the dry weights of the bark samples were reduced on the average by 17 percent, the total nitrogen was reduced by 25 percent, and the nitrogen per gram by 11 percent. In no. 3, the dry weights of the bark were increased on the average by 50 percent, the total nitrogen was increased about 2 percent, and the nitrogen per gram was reduced by 22 percent.

Considering the twig as a whole, the rings at the bases of the defoliated parts distinctly reduced both their total nitrogen content as well as that per gram of dry weight.

Discussion

The results recorded in this paper are not in accord with those reported by Hibino (1917), who found that the proteins and ash contents of twigs of *Cornus controversa* were higher in the twigs ringed to the cambium than in the checks. Those ringed through the sapwood showed still higher protein, but lower ash than the checks. The protein contents in percentages of dry weight were 5.75, 6.31, and 7.81 respectively, in the check twig, in that ringed to the cambium, and in that ringed through the sapwood. The ash contents were, respectively, 1.45, 1.75, and 1.32. Evidently but very few twigs were analyzed, and since comparisons were made between different individuals, and since their composition was not known at the beginning of the experiment, the final differences may have been due to individual differences existing from the beginning. As discussed in connection with table 7, I have found the nitrogen contents of stems of privet to vary much more than those of leaves. Stems from similar plants were commonly found to vary in nitrogen content between 8 percent and 10 percent, and extremes were found with 6.39 percent and 14.96 percent nitrogen, a variation of over 100 percent.

When it was found that some nitrogen evidently passes a ring, as indicated in table 3, it seemed possible that on long standing a ringed stem might accumulate more nitrogen than a normal stem, since probably little or none of that passing a ring is ever carried back. The stems in Hibino's experiments had been ringed in July and were sampled in January. The data presented in table 2, however, indicate that leaves from a stem that had been ringed over a year may still have a lower nitrogen content.

Combes (1912) has reported ash analyses of leaves from ringed and normal stems of various woody plants. Expressing the ash as percentage of dry weight, the leaves from the ringed stem had lower ash contents in every case, and when expressed as percentage of fresh weight, all but two of the seventeen showed lower ash contents. When expressed as total ash or as ash per leaf, with the exception of *Pinus excelsa*, which in every

sample had more ash in the leaves of the ringed stems. there were no regular differences between the unringed and the ringed stems. As no leaf areas were reported, no definite conclusion can be drawn as to whether the ringing had altered the actual ash content.

It is very evident from the data presented in this paper that ringing has hindered the movement of nitrogen and ash constituents into the tissues above the ring. This has been found to occur in all the plants studied, including peach, cherry, lilac, and privet. Experiments were also tried with chestnut, maple, and pear, but these were done so late in the year that no increase of nitrogen was evident even in the check stems.

Whether the results can be considered as proof that nitrogen and other mineral nutrients move up through the phloem and not through the xylem with the water, may still be open to doubt. It is very probable that the rate of transpiration is usually less from the ringed stems. If there is an actual mass flow of water in the transpiration stream without passing through filtering membranes, a flow as through a pipe in an ordinary water system, if nutrients are carried in this stream and the associated living cells do not remove them, then the rate of transpiration would, of course, influence the amount of nutrients reaching the transpiring tissues. So far as I am aware, however, nothing as yet has been published showing conclusively that there is such a "transpiration stream" in plants or that the rate of transpiration directly determines the amount or rate of nutrient movement to the transpiring tissues. The one preliminary experiment reported in table 7 would indicate that transpiration is not an important factor directly determining the distribution of nutrients. The data presented by Muenscher (1922) give perhaps even better evidence that transpiration does not directly influence the distribution of nutrients. Though his experiments were chiefly directed to determine the influence of transpiration on the absorption of nutrients, they offer evidence that it does not influence nutrient movement after absorption, for, if the removal of nutrients from the roots were hastened by transpiration, this hastened removal should cause greater absorption for the plant as a whole and should reduce the ash in the roots, the absorbing organs. He found, on the contrary, that, when transpiration was altered by light and shade, plants with high transpiration rates showed an ash content of the roots distinctly higher than those with low transpiration, when measured as total ash or as percentage of dry or green weights, and an ash content of the tops somewhat lower in percentage but higher in total amount. Of course, in neither case do the ash contents indicate the amounts present in the conducting system. This high ash content in the roots is probably due to their increased content of organic matter, and, in fact, it is difficult to arrive at any definite conclusion with respect to the effect of transpiration on the distribution of nutrients in experiments of this type in which the organic content of the tissues is not under control.

Furthermore, even though transpiration above a ring may have been reduced, the high carbohydrate content of the tissues above the ring, as well as the ring itself, would tend to prevent the removal of nutrients once they got up past the ring. This failure in removal would tend to offset any possible slower absorption that might result from decreased transpiration.

Aside from difficulties in interpretation, resulting from possible differences in transpiration, there is the difficulty that the xylem itself may have been altered by the ringing. Several writers (Daniel, 1906; Combes, 1912; Higgins, 1919, and others) have offered evidence that tyloses or gum may plug the xylem vessels in the region of a ring or other wounds, but in none of those instances that have come to my attention have precautions been taken to protect the exposed xylem. In nearly every one of my own experiments in which the exposed xylem of small twigs has not been immediately covered with a layer of melted paraffin, the leaves above the ring have shown withering sooner or later. Those that were protected rarely showed withering within the time of the experiment, and in no case were samples taken from stems that showed withering. In other experiments, however, which are not yet completed, I have obtained indications that rings even when protected may increase the resistence of a stem to the flow of water. Even if it were conclusively demonstrated, however, that ringing resulted in a partial plugging of the xylem, it could not be said with assurance that such plugging is the reason for the lower nitrogen and ash contents in the tissues above a ring.

Summary

Experiments are reported showing the effects of ringing a stem on the upward transfer of nitrogen and ash constituents. Data from such experiments with privet, peach, and lilac are presented.

The data show that a ring distinctly hinders the movement of nitrogen and of ash constituents into the leaves above the ring, either when the ring is made in the spring before the leaves open and the new xylem is laid down, or when it is made in the summer after they have opened and the new xylem is partly or fully formed.

When sodium nitrate, with or without other nutrient salts, is added to the soil, the nitrogen and ash contents of the leaves from unringed stems increase to a much greater extent than do those of the leaves from ringed stems. This is true whether data are expressed per unit of dry weight, per unit of leaf surface, or as absolute quantities.

The stems also were analyzed to determine whether or not the low nitrogen content of the leaves could be accounted for by accumulation of nitrogen in the stems. These analyses, whether of the entire stem or of the wood and bark separately, showed a lesser content in the ringed stems.

A single experiment was tried in which an attempt was made to eliminate

the influence of altered transpiration or of change in carbohydrate content. In this experiment, evidence was obtained indicating that the low nitrogen content above a ring is not due to a lessened transpiration or to a changed carbohydrate content.

The data cannot be considered as conclusively proving that nitrogen or other nutrients move upward primarily through the phloem and not through the xylem, for the treatments may have altered the xylem tissues. Aside from such a contingency, however, these data from ringing experiments offer strong evidence indicating that nutrients are carried chiefly in the phloem.

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THE INFLUENCE OF PLANTS ON THE AIR IN HOUSES

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THE PROBLEM

The experimental work reported in this paper deals with the percentage of carbon dioxide in the air of a city greenhouse at various times of day. The discussion deals with the relation between the presence of plants in rooms at night and the comfort and safety of persons occupying the rooms.

It has been known [1] for several years that ventilation of occupied rooms is necessary to keep down the temperature, secure cooling movements of the air, and maintain a low humidity, and not to let out air containing a high percentage of carbon-dioxide and admit that having a low carbon-dioxide content. It is possible also that one good effect of ventilation is the removal of injurious organic matter[2], and it is certain that it is beneficial in preventing the continued multiplication of pathogenic organisms.[3]

In the light of this information, it seems strange that so little attention has been paid to the nature of any effects that the presence of plants may have on the air of rooms occupied for sleeping purposes. There is a widespread popular belief that plants in a room at night are injurious to persons sleeping in the room. The recent experience of one of us in a large hospital where an attendant is kept busy for several hours every day removing plants from the rooms at night and replacing them in the morning has directed attention especially to this belief.

While most textbooks on botany and physiology simply ignore the subject of the relation between plants and people in sleeping rooms, a widely used school chart bearing the statement that it has been revised by the dean of the college of agriculture in a tax-supported university says:

> We can understand why plants should have no place in sleeping rooms at night for when light is withdrawn, the absorption of carbon dioxide stops and the plants give off this poisonous gas.

Since Winslow [4] has found that air contaminated by the breathing of human beings until it contained an average of 20 to 60 parts of carbon-dioxide per ten thousand parts of air, five to fifteen times the normal amount,

[1] Winslow. Sci., n. ser., **41**: 625–632. 1915.

[2] Since the above was written the writers have seen the Report of the New York State Commission on Ventilation (Dutton and Co., New York, 1923) which states (p.8) that the weight of scientific evidence is against the existence of organic poisons in respired air.

[3] *Cf.* Nolte. Ann. Mo. Bot. Gard. **1**: 47–80. 1914.

[4] *Loc. cit*; also Rept. N. Y. State Com. on Ventilation cited above.

produced no bad effects on the occupants of the room so far as physiological and psychological tests show, and since Pettenkofer [5] long ago showed that carbon-dioxide is quite without effect on human beings in the highest concentration that it ever attains in occupied rooms, our attention has been directed especially to the percentage of carbon-dioxide, during the day and night, in a room containing a large number of growing plants.

The work was carried on in the greenhouse in Volunteer Park in Seattle. The room in which the samples of air for analysis were secured was well filled with plants, so that the total leaf area exposed was large in proportion to the volume of air. The investigation was carried on in July when the plants were growing actively. The room contained a considerable number of tropical plants whose growth was especially rapid and whose metabolism was thus very active. The plants were potted in rich soil fertilized with organic fertilizers every two weeks, and no doubt microorganisms in the soil were active agents in the production of carbon-dioxide. The room had top ventilation of the type commonly provided in greenhouses.

Experimental

Hesse's method as reported by Scott [6] was utilized for determining the carbon-dioxide content of the air. The necessary apparatus together with the standard solutions were taken to the greenhouse, where all analyses were performed. This eliminated the transport of samples to the laboratory and facilitated the checking of data. The results were calculated for dry air at standard conditions and are reported in the number of parts of carbon dioxide per ten thousand parts of air.

All precautions were taken to keep reagents and samples of air collected from contamination by carbon-dioxide. The samples were secured by emptying a large Erlenmeyer flask, previously calibrated, of distilled water saturated with the air to be sampled and having the same temperature. As soon as the flask was emptied, it was immediately corked and taken to the titration bench where standard barium hydroxide was added and the flask was shaken for ten or more minutes. The excess of alkali was titrated with standard oxalic acid, after washing down the sides of the flask with boiled distilled water. The solutions were admitted to the flask through a hole in the stopper of the flask. The data obtained are given in table 1.

Discussion

It is evident that certain factors tend to decrease the amount of carbon-dioxide in the air of this greenhouse, while others tend to increase it. Under the first head come (*a*) the green plants which use carbon-dioxide in photosynthesis during the day but not at night, and (*b*) ventilation, including

[5] Quoted by Winslow, *loc. cit.*

[6] Standard methods of chemical analysis, p. 728.

wind, which creates more or less draft through the room. Under the second head come (*a*) the presence of people, producing carbon-dioxide by their respiration, (*b*) the green plants likewise producing carbon-dioxide whether in light or in darkness as the result of respiration, and (*c*) the micro-organisms also producing carbon-dioxide at all times.

TABLE 1. *Parts per Ten Thousand of Carbon Dioxide in Air of a Greenhouse at Various Times of Day* *

Time		Parts CO_2 in 10,000
1921		
July 12	4:10 P.M.	10
13	8:30 A.M.	6
13	3:10 P.M	8
14	3:00 P.M.	5
15	10:30 A.M.	9
15	5:20 P.M.	5
16	12:50 P.M.	6
18	6:45 A.M.	4
18	7:00 P.M.	4
19	7:05 A.M.	5
19	7:00 P.M.	6
20	2:45 P.M.	4
21	6:45 P.M.	4
21	8:50 P.M.	3
22	7:00 A.M.	3
22	10:50 A.M.	4
22	6:15 P.M.	5
22	9:50 P.M.	5
23	1:30 P.M.	6
24	1:00 P.M.	5
24	7:45 P.M.	6
25	1:45 P.M.	6

* Calculated for dry air at standard conditions.

It might be expected that during the day photosynthesis, using carbon dioxide, and respiration, liberating it, would approximately neutralize each other, and that thus the plants would have little effect on the net amount of carbon dioxide in the air; while at night, photosynthesis being stopped and respiration continuing, the carbon-dioxide content of the air would be increased. On this basis, one should expect a greater percentage of this gas in the morning when the plants have been several hours in darkness than in the afternoon when they have been in light for some hours. The tests, however, show just the reverse of this. The average of the seven carbon-dioxide determinations made in the forenoon was between 4 and 5 parts per ten thousand, and that of the twenty in the afternoon, between 5 and 6 parts per ten thousand.

It does not seem probable that the activities of the soil organisms could account for this difference, though it is known, of course, that the rate of respiration in many plants is increased by intense illumination. Ventilation might either increase or decrease the carbon-dioxide content of the air in the greenhouse, but, since it was at the time of all tests higher than that of normal air, it is evident that it never increased but always

tended to decrease it. There seems to be no reason to suppose that ventilation was more effective in decreasing carbon-dioxide content at night than during the day.

The only remaining cause that could account for the accumulation of carbon-dioxide in the daytime was the presence of people visiting the greenhouse. The number of visitors varied greatly on different days and at different times of the day. A very high percentage of carbon-dioxide was found at one time when a gathering of florists and botanists inspected the greenhouse and it was crowded during the entire afternoon, and also on another occasion when a large number of school children came to see the plants. The irregularity in the number of visitors is undoubtedly the cause of the wide discrepancies in the values found at different times. The greenhouse was closed to visitors at 6 P.M.

Since, according to Winslow's data, the amount of carbon-dioxide could be increased to a point five to fifteen times as great as the maximum found in this greenhouse without injury to people, and since the amount of leaf area exposed here was very large in proportion to the volume of the air, while it is very small where a few plants are kept in sleeping rooms, it would seem that the amount of carbon-dioxide produced by a few plants in a sleeping room would not reach the danger point for the occupants or even approach it. Any danger must rest on other grounds, such as high temperature, high moisture content of the air, or some other factor.

Summary

1. Determinations of the carbon-dioxide content of the air in a greenhouse indicate that the concentration of this gas did not at the time of any determination reach a high enough concentration to be injurious to human beings.

2. Under the conditions existing in this greenhouse, the effect of plants in increasing carbon-dioxide content is negligible in comparison with the effects of the people who visit the greenhouse.

3. Any bad effects that may at any time have been experienced from the presence of plants in reasonably ventilated sleeping rooms must rest on some other basis than carbon-dioxide production.

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THE POTENTIALITIES OF A CELL [1]

CHARLES E. ALLEN

I

On the basis of evidence now at hand, it is generally agreed that the flagellates represent the ancestral group from which came most existing plant and animal phyla. More specifically, it is to the free-living flagellates that we look, and, as concerns most groups of plants, to the pigmented forms, for the nearest contemporary approach to phylogenetic origins. It is not to be forgotten that the flagellates now living represent the outcome of as long an evolutionary development in point of time from any common point of departure as do the angiosperms; and that only very cautiously may conclusions be based upon present-day flagellates as to the nature of the forms from which higher organisms have been derived. With the latter fact in mind, however, certain suggestions of a very general nature seem fairly safe.

One notable characteristic of the pigmented flagellates is a *plasticity* in form and function. The familiar Chlamydomonas, for example, may take the form of a flagellate cell; of an amoeboid cell which ingests organic food in an "animal-like" fashion; of a non-motile cell, divisions within whose wall result in the formation of a temporary colony; of a flagellate gamete; of a non-flagellate zygote, differing from the "vegetative" quiescent form in the nature of its wall and of its secretions; and of a palmelloid colony.

Myxochrisis paradoxa may be selected as another illustration. This brown flagellate, according to Pascher's description, appears as cells, single or in small groups, with rigid walls, one-, two-, or several-nucleate; as naked, one-nucleate flagellate cells; as amoeboid cells; as filar plasmodia formed by the partial union of amoebae; as "true" plasmodia, resulting either from the growth of single amoebae accompanied by nuclear division, from a fusion of several or many amoebae or plasmodia, or from a combination of the two processes; and as encysted plasmodia which by division form temporary colonies. In each phase, some individuals lack the characteristic brown chromatophores. Cell division seems to occur in any phase except possibly the amoeboid. Different phases are characterized by holophytic or holozoic nutrition, or by a combination of the two methods.

These instances appear to be fairly typical. Many species of both green and brown flagellates are described whose visible cellular organiza-

[1] Address of the retiring president of the Botanical Society of America, read at Boston, December 28, 1922.

tion is from time to time profoundly modified, the transition from one phase to another seeming to occur on relatively slight provocation. Such plasticity is especially striking in view of the present very imperfect knowledge of most species; indeed, it is not certain that all the phases into which any one species may pass have yet been recognized. A comparable plasticity, it may be observed, characterizes other protistan groups; recent reports, for example, indicate the occurrence in bacteria of comparable and quite remarkable modifications in cell size and cell structure. The pigmented flagellates are selected for present mention because of their apparent phylogenetic significance.

In entering upon and in passing through a particular phase (such as the amoeboid), the cell of a flagellate race performs certain functions. The ability to perform each of these functions results from the chemical and physical make-up of the living matter or of some of its parts. Since all known living matter is organized into cells, the possibilities or *potentialities* that inhere in the constitution of the living matter may be spoken of as the *potentialities of the cell.* Each function that the cell or one of its parts performs is the expression of a potentiality.

The expression of certain potentialities, particularly of those concerned in the more fundamental processes, is common to two, or to several, or to all phases. But the potentialities manifested in one phase differ, as a group, from those manifested in another phase. Smaller differences exist between different stages, for example, of the amoeboid phase, as to the particular group of potentialities momentarily functional, or as to the degree or manner of expression of particular potentialities. Some potentialities can be expressed only in conjunction with certain others; some are mutually exclusive; and from the latter fact arises the possibility of distinct phases in the history of the race. A list of the potentialities of any cell, therefore, would be in effect a list of all the functions,the capacity for which is inherent in the living matter of that cell.

A distinction may be drawn between an actual change in the form and activities of a cell—as the transition from a flagellate to an amoeboid form—and the origin from the cell, by division, of cells of a different form—as in the production from a plasmodium or a multinucleate cyst of numerous flagellate offspring. In either case, the *race* passes from one phase to another; the individual *cell* may not. Obviously a cell can transmit to its offspring only the potentialities which it possesses; and hence the potentialities of all phases possible to the race are possessed by any cell in any phase; even though the cell, in a particular phase, is itself debarred from expressing some of its inherent potentialities.

This fact, that a cell possesses potentialities which, having reached a certain phase, it can not itself express although it can transmit them, calls attention to the widely recognized dual constitution of living matter. The heritable potentialities arise from what may be called, non-commit-

tally, some *fundamental features* of cellular organization. The persistence of these fundamental features is consistent with marked alterations in the form, the internal structure, and the functions of the cell. The persistent, heritable features of organization are undoubtedly in large measure, in nucleated cells, especially characteristic of the nuclear substances, the cytoplasm being the medium through which from time to time varied sets of potentialities are expressed. However, the division of functions is probably not quite so sharp as this statement would imply; for some few, at least, of the hereditary potentialities of the cell seem to depend upon persistent features of the constitution either of certain parts of the cytoplasm, such as plastids, or of the cytoplasm as a whole.

The passage of the cell from phase to phase is conditioned by stimuli. This is true at least to the extent that, when a change is to take place, surrounding conditions determine just what that change shall be. Whether *some* change in the activities of the cell would occur if the environment remained unmodified, no one can say. Indeed, the activities of the cell necessarily modify its environment; so that an unchanging environment for a living cell is unthinkable. Experiment shows, however, that a very large proportion of the processes of change that constitute life are or can be brought about by environmental changes; and that, as between two or more possibilities at any point in the story, the environment largely determines which alternative shall prevail. To this extent, a particular potentiality may be described as the power of responding, by a certain activity, to a definite stimulus or group or class of stimuli. In recognition of this point of view, various writers have described all the characters of an organism as responses.

But this statement, like most generalizations, may be too broad. That the organization of the living matter not only establishes certain potentialities, but, in conjunction with the environment, plays a part in determining the sequence of expression of those potentialities, is suggested by various facts. One such fact, already cited, is that a cell, in any particular phase, can not pass indifferently into any other phase whose potentialities it possesses. Limitations of this nature result in the appearance of something approaching a life cycle. For instance, from the multinucleate sclerotium of Myxochrisis may come, after cell division, thick-walled quiescent cells, flagellate cells, or amoeboid cells; a quiescent cell, forsaking its wall, may become flagellate or amoeboid; and a flagellate cell may pass into the amoeboid phase. In spite of a considerable range of alternatives, the story moves in a certain general course; for an amoeboid cell does not, as a rule at least, reverse the order and take on the flagellate or the quiescent form. It is too early to say what further variations of the history unusual conditions may bring about; but a general tendency toward something like a cycle seems manifest. As there is a plasticity in form and function, so there is a plasticity in the sequence of forms and functions; but both

types of plasticity are measurably limited. The tendency toward a certain succession of phases sometimes, no doubt, results from limitations imposed by the conditions of the particular present phase. Thus, a plasmodium or a multinucleate cyst, of whatever species, can not, or at least usually does not, return to the uninucleate condition except by a division into uninucleate cells, in which process the identity of the mother cell disappears. Here a different course of events is clearly difficult or impossible. But the limitations noted upon the sequence of quiescent, flagellate, and amoeboid phases have a less obvious physical basis. Such limitations, like the potentialities whose expression they affect, are inherited. Like the potentialities, they seem to have their basis in the fundamental organization of the living matter.

Other heritable characters that may be considered directing or limiting tendencies are polarity and the various specific types of cellular symmetry. In the same category perhaps belong some of the "inhibiting" and "lethal" factors of genetic analysis. It appears, then, that the fundamental organization of living matter, besides giving rise to a great variety of potentialities, likewise in some degree limits and directs the order of expression of those potentialities. The question is still open whether the living matter, apart from the interaction of the environment, is able to do more than limit and direct—namely, to *initiate* the expression of any of its own potentialities.

Especially characteristic of living matter is one potentiality of far-reaching significance—that of undergoing changes in its fundamental features. From time to time, and under almost entirely unknown conditions, the specific character of the living matter is altered; some of its potentialities may be modified, some lost, or new ones acquired; and thus a new race is born. The possibility of changes in fundamental organization is limited; evolution can not move in any conceivable direction. The directions in which it may move are themselves determined by the constitution of the living matter. A question now arises paralleling that already asked concerning the expression of specific potentialities: How far are evolutionary changes themselves responses to stimuli, and how far the necessary result of the cell's constitution? To what extent, if at all, would new races arise if the relatively persistent features of the organization of living matter were uninfluenced by the environment? This question has been variously answered, but it is doubtful if any of the answers is more than an interesting guess.

II

Each of the types of evolutionary change just suggested—loss, gain, and modification of potentialities—is illustrated by flagellates that have become adapted to a strictly or mainly holozoic, saprophytic, or parasitic existence. Such highly developed parasites as the Trichonymphidae, for example, have lost the potentialities concerned in photosynthesis. Cor-

related with this loss, whether simultaneous or subsequent, was the disappearance of chromatophores. In other ways the range of potentialities has been limited, so that the cell can assume (so far as known, with the possible exception of one species) only the flagellate form. The plasticity of the pigmented species, as shown by the number of phases possible to the organism, has been reduced.

Some of the changes accompanying the complete loss of potentialities consist in the modification, rather than the loss, of certain powers of response. The specialization in adaptation to a parasitic mode of existence implies that the race remains under a greater variety of conditions in a phase suited only to parasitic nutrition. That there has been a real change in responsive power is shown by the great difficulty of keeping these organisms alive in any habitat other than that to which they are so narrowly adapted.

On the other hand, new potentialities have appeared. Without much doubt the adaptation to a parasitic existence has involved the acquisition of new powers of digesting and assimilating the types of nutrients now available. New potentialities certainly show themselves in the development of intracellular structures, notably the elaborate neuromotor apparatus recently described in detail by Kofoid and his students. It is quite possible, too, that the loss of the power of passing into certain phases is in a measure compensated for by an increased power of responding by relatively small modifications to the minor environmental changes which are all that an internal parasite ordinarily encounters during its active life.

Comparable changes have occurred in the evolution of other protists from flagellate ancestors. Each phase in the history of a slime mold such as Stemonitis, except that of the sporange—walled spore, swarm-spore, amoeba, microcyst, plasmodium, sclerotium—is duplicated, or simulated, in the life cycles of numerous flagellates. So many phases persist that, until the course of descent of the myxomycetes is known, it will be impossible to say what, if any, potentialities have been lost. On the other hand, new potentialities, leading to the formation of a rather complex sporange and capillitium, have been acquired. It is possible, however, that some of the potentialities concerned in sporange-formation are not really new to the myxomycetes, but are old ones which have been modified in time of appearance, in degree, or in duration. In large measure, too, there have been modifications in responsive power which affect the duration of the various phases.

The life cycle of Stemonitis consists of a series of phases, still with marked possibilities of substitution, suppression, and extension, but none the less following one another in fairly definite order and in the main incapable of inversion. The tendency operating in the pigmented flagellates toward some sort of sequence of stages seems to have become more effective. Thus the change in fundamental organization which has diminished the flagellate

plasticity involves, in the case of a slime mold, not so much a loss of particular potentialities as the organization of the expression of potentialities into a more nearly rigid series.

III

The changes involved in the evolution of coenobic species may be similarly outlined. No sharp line exists between temporary colonies which, for the period of their existence, are coenobes, and the more durable colonies that characterize some flagellates as well as many derived forms. So far as concerns the potentialities upon which the formation of a coenobe depends, perhaps little strictly new appears at this evolutionary level, because the prototype of each method of colony-formation (instance those of Scenedesmus, Hydrodictyon, Tetraspora, and Spirogyra) is to be found among the flagellates. Changes have occurred which render the cell less likely to respond to environmental changes by separation from its fellows. The phases characterized by an independent existence have been subordinated. Especially is this true of the flagellate phase, which in some lines, such as the Zygnemaceae, has dropped entirely out of the ordinary life cycle. That a particular phase does not regularly appear in the life cycle is not, to be sure, proof of the total loss of the potentialities necessary to its appearance. This fact is shown by the ability of the cells of Tetraspora and Stigeoclonium under some conditions to pass into an amoeboid phase in which they are capable of holozoic nutrition.

The type of coenobic colonial plant that proved best adapted to further evolution on this planet involves the continued close contact of sister cells after each of a series of divisions. The existence of the colony, in general, depends upon the formation of a persistent rigid layer between the sister cells at the time of, or shortly after, their formation. This layer may in some cases constitute the final thickness of the partition wall. Much more commonly, probably, it is supplemented by the deposition of additional layers on either side; the original layer then corresponding, in history, position, and function, to the middle lamellae of the larger green plants. The formation of a persistent partition wall depends upon the ability to secrete, under definite conditions, a certain substance or certain substances. Sometimes the original layer is easily ruptured, as in the common yeasts. But in the evolution of persistently colonial plants the middle lamella, if once fragile, has become more stable.

In a coenobe, such as Spirogyra, every vegetative cell has, throughout its active life, the full range of potentialities of every other cell. Any cell may remain vegetative, grow, and divide, or may become a gamete or an azygospore. When, in a coenobe, a cell responds to particular stimuli by taking on the characters of a gamete or of a spore, it becomes differentiated from the vegetative cells; but it can still transmit its full original equipment of potentialities to its offspring although it may itself be debarred

from expressing certain potentialities. A similar limitation, it has been seen, may be experienced by a one-celled organism when it has passed into particular phases. It follows that there is no difference in inherent potentialities as between the constituent cells of a coenobe. Harper has shown that in Hydrodictyon the potentialities of the equipotent cells determine, with the interaction of the environment, the character of a comparatively large and definitely constituted plant. He has shown the same to be true of Pediastrum in which some differentiation of cells appears, in well as of so complex a plant as Dictyostelium with its extensive cellular differentiation.

IV

The transition from coenobes to plants with differentiated cells, like that from unicellular organisms to coenobes, is gradual. Differentiation in structure and function implies the expression by different cells of different groups of potentialities; it results directly, as is obvious in the filamentous algae and fungi, from differences in the conditions surrounding the respective cells. All the cells of the plant are still equipotent; and any cell possesses all the potentialities of all the cells—it is totipotent—as is shown by the fact that any cell, at least while young, may give rise, in one way or another, to a complete new plant. The potentialities common to all the cells are, as it were, under the influence of the environment, sorted out and arranged into alternative life cycles; or it would be better now to say *life histories*, since some of the alternatives may not lead to reproduction and hence to a repetition of the story. But the differentiation takes place in the life of each cell. All the cells are, at the start and throughout at least most of their history, alike in their potentialities; all are, in Weismann's phrase, potentially immortal.

There may come a time in the history of a vegetative cell when it can no longer divide, and hence can under no circumstances originate a new plant. Obviously the power of reproduction is lost in the changes which precede the death of the cell; but it is possible that this loss, in some types of cells, anticipates the appearance of any degenerative changes. Certain of the alternative histories may, therefore, lead in time to a condition in which the cells upon which those alternatives were forced lose their immortality. One-celled organisms may likewise, in some environments, pass into a condition in which reproduction can not occur. The possibility of a condition of this sort is thus not new to the constituent cells of a many-celled organism; but in the latter case some of the cells necessarily lose their reproductive power as a result of their position in the plant.

Some degree of differentiation must result from a mere increase in size of the colony; for the larger the colony the more varied are the conditions to which its different cells are exposed. If, however, cellular differentiation were a function of size alone, Rhizoclonium and Draparnaldia would

be about equally differentiated. Differentiation beyond its most elementary stages evidently depends also, and more largely, upon an increasing susceptibility of cells to relatively slight differences in stimuli; the increased susceptibility brings about the expression of noticeably different potentialities by cells differently situated. Probably also quite early in evolutionary development—very certainly on a large scale at a later stage—there is an increase in the range of potentialities at the disposal of the cell, and hence also in the variety of possible life histories.

The secretion of a persistent partition wall between sister cells makes a colony possible, but of course does not determine the form of the colony. The form, whether unbranched or branched, filamentous or plate-like, depends in part upon the expression of various potentialities concerned with the relative growth of each cell in its respective axes and with the plane or planes in which division occurs. Some of these potentialities are among the new evolutionary developments; and, since species differ in these potentialities, they differ in their characteristic forms.

V

When, in evolutionary course, the potentialities appeared whose expression resulted in the development of a massive plant—one with cell divisions in more than two planes—the possibility of differentiated cellular development led to the formation of tissues. The internal cells of a massive plant are shielded from the immediate influence of conditions outside the plant, although indirectly, of course, still much affected by those conditions. The internal cells are subjected to an environment, a very important and immediately influential part of which consists of the surrounding cells. The variety of stimuli, including pressures and tensions and electrical and chemical changes, which act upon different cells of the plant is thus greatly increased, and there is made possible an increase in the variety of cellular differentiations. It is to be expected, then, that, quite apart from the acquisition of new potentialities, the arrangement of potentialities into alternative life histories should be carried further than in a filamentous or plate-shaped plant whose cells, while by no means uninfluenced by stimuli proceeding from neighboring cells, are all alike in touch with the world outside. Any increase in the size and complexity of the plant magnifies the importance of the interaction of its cells. In a large measure, as Goodrich has expressed it, the higher organisms have gradually substituted internal for external stimuli.

Every newly formed cell of a massive plant is embryonic, in the sense that it is capable of division. In general, an embryonic cell has certain structural characters; but the presence or absence of these characters does not affect its essentially embryonic nature. By the division of the embryonic cells in a primary meristem, followed by later divisions of some of their still embryonic derivatives, all the cells are provided which are to

constitute the tissues of the plant. The individual destiny of the cells formed in the meristem is evidently in no sense predetermined. Whether any such cell is to become a highly differentiated element of the xylem, phloem, or epidermis, or is to retain its embryonic character as an element of the meristem, cambium, pericycle, or cortex, depends upon its position, and therefore upon the stimuli which affect it. The same statement holds for the cells formed in the cambium or in any other embryonic region. A material alteration of conditions, as by a wound, by the incursion of a parasitic fungus, or even by a change in the position of the plant, may result in radically changing the course of development of a cell, even after it is well started upon the processes of differentiation. The life history of the cell, under the changed conditions, is shifted to a course quite different from that which a cell in its position would have followed under ordinary circumstances. The facts of development under both ordinary and exceptional conditions thus indicate that all the cells of a complex as of a simpler plant are, at least while embryonic, equipotent and totipotent.

A special case of a shift in the life history of a cell or of a group of cells as a result of changed conditions is seen when new organs or new plants are produced by regeneration. So extensive is the power of regeneration in bryophytes that one is almost tempted to say that any cell, at any time during its active existence, can give rise to a complete plant. In the vascular plants the power of regeneration is widespread also; witness the varied forms of vegetative multiplication, regeneration from leaves, the production of adventitious shoots and roots. In some cases regeneration involves the dedifferentiation of cells already well advanced in their developmental history. But it seems clear—though perhaps not yet rigidly demonstrated—that in a vascular plant each type of differentiating cell may reach a point in the course of its development beyond which, although it is still alive and functional, dedifferentiation and division are impossible. It is evident that such a state has been attained by "mature"—that is, dead—tracheids and vessels, as well as by the still living but enucleate cells of sieve tubes; but it seems to be true that in these and in other tissue cells there are conditions—among others, much thickened walls—which effectively inhibit division even before differentiation has run its full course.

The statement of the primary totipotence of all the cells of a plant must be modified by the admission that occasional irregularities in the working of the mitotic mechanism may result in an unequal distribution of potentialities. There is both cytological and genetic evidence that these mitotic accidents occur, perhaps not infrequently. But the possibility of such disturbances does not affect the validity of the general law of equi- and totipotence.

Among the metazoa an unequal distribution of potentialities is perhaps regularly affected in some cases; for example, by a discarding of part of

the chromosomal substance, as in certain species of Ascaris, or, as in some insects, by the inclusion in certain cells of particular cytoplasmic substances. If, and so far as, these occurrences effect a differential distribution of cellular potentialities, rather than something like a stimulus to a particular cellular development, they constitute deviations from the condition of primary totipotence which is probably as truly the general rule for the cells of animals as it is for the cells of plants.

The potentialities whose manifestation marks each particular type of cell in a vascular plant come to expression—under ordinary conditions—in a definite order. This orderly development seems to be determined in part by an inherited tendency to pass through a definite sequence of phases—a tendency which now applies to all the alternative life histories upon one of which the cell has embarked. But the orderly development is also partly dependent upon a progressively changing environment, which consists largely of other cells among which the one in question is firmly held. The importance of the environment is shown by the fact that, although the course of development of any cell of a massive plant seems to be less easily diverted to an alternative course than is that of a protist or of a cell of a coenobe, the cellular life history is, nevertheless, not rigidly fixed. In some cases, and for a limited time at least, it is subject to reversal—dedifferentiation. It is capable for a considerable time of being greatly modified by the onset of changed conditions. The stimulus that causes the formation of a gall may determine the expression of potentialities that otherwise would not be manifested either in the organ affected or in any other part of the plant. Thus the whole range of cellular potentialities may not be exhibited in a plant living under what we call "normal" conditions. To comprehend the extent of this range it is necessary to observe the plant under all conditions, including pathogenic, that it is capable of enduring.

In the course of the development of an embryonic cell into a differentiated tissue element, the structural peculiarities that usually characterize embryonic cells are modified. But the cell may still remain essentially embryonic. Next, perhaps, it becomes itself incapable of taking on characters other than those proper to the cells of the tissue of which it has become a part; but, so long as it retains the power of dividing, it can still transmit to its offspring its full initial complement of potentialities. Finally it may, and in many cases probably does, while still alive and otherwise functional, lose all power of division, and therewith its embryonic character.

The story that is presented, therefore, in ontogenetic development is that of a plant, simple or complex, composed of totipotent cells all beginning life with the power of transmitting their totipotence to their offspring. However complex the plant, many cells retain this power, remaining embryonic until they divide or become moribund. Others, undergoing marked changes in structure and in function, may in time lose their totipotence and their power of division. But so long as they can divide, they can give

rise to totipotent offspring. In this history there appears neither a fundamental distinction between "germ" and "somatic" cells, nor a progressive distribution of potentialities during ontogeny to differentiating groups of tissue elements. These two conceptions, it may be said in digression, have found some seeming justification in animal embryology; partly by the unequal distribution of particular nuclear or cytoplasmic substances already referred to; and especially by the fact that tissue development in the more complex metazoa involves a progressive differentiation extending through a series of cell generations. The history in these organisms, under ordinary conditions, from obviously embryonic cell to fully differentiated tissue element includes the terms of existence of a succession of cells, being in this respect somewhat comparable with the less strictly defined life cycle of a many-phased flagellate; whereas in a plant the corresponding history is mainly or wholly included within the lifetime of a single cell. But despite this important difference, it is probable that the fundamental ontogenetic processes of the metazoa do not differ in principle from those here outlined for the vascular plants. The culture of isolated animal tissues promises to supply important evidence upon this question.

The evolution of complex plants seems to have involved the loss of some of the potentialities that characterized their flagellate ancestors. The ability to revert to a flagellate phase, as shown in the formation of swarm-spores or motile gametes, has itself been lost—so far as we know—by the cells of conifers and angiosperms. It is noteworthy that in many ascending series, including those which led to the seed plants, this power was so long retained, even after incalculable ages of life upon land. The ability to pass into a naked amoeboid phase, still present in some of the Chlorophyceae, has also—again so far as we know—been lost by the cells of the more complex green plants. But if a few potentialities may be shown to have been lost, more have been gained. The widely varied types of cells found in the usually developed tissues of an oak, not to mention those of pathological growths, represent a vast number of potentialities which every newly formed cell of the tree possesses. Few of these potentialities, apart from those concerned in the more fundamental activities, were possessed by the oak's flagellate ancestors. The overwhelming majority have been added to the heritage of the cell. The acquisition of numerous new potentialities has characterized the evolution of the cells of complex organisms, both plant and animal.

The responses which consist in the seriated expression of potentialities characteristic of the cells of any tissue are adjusted with remarkable delicacy to what must be minute differences between the stimuli that incite them. This delicacy of adjustment to varying stimuli is apparent if one considers two adjoining cells, starting with the same inheritance; one becoming, perhaps, an element of the cortical parenchyma, another a bast fiber or a sieve-tube element. It follows that, besides many new po-

tentialities, the cell has acquired in the course of its evolution a susceptibility to differences in stimuli probably far beyond that possessed by any algal cell. This is another important feature in the evolution of complex organisms.

The net result of the delicately adjusted responses to almost innumerable stimuli—including particularly those due to the presence and activities of other cells in the same plant—is not only the orderly development of each cell but also a coördination in the development of all the cells. The coördinated responses of individual cells bring about "regulated" growth and development. The totality of cellular development, thus coördinated, is called the life history of the organism. It is not surprising that some investigators, viewing the history of the organism as a whole rather than as made up of the histories of individual cells, and bewildered by the complexity of development thus considered, have expressed their despair by the coining of magic words or phrases, intended to express the inexpressible, to explain the inexplicable. This is a needless confession of hopelessness. It is reasonable, and immeasurably more profitable, to assume that regulation can be explained, by a painstaking analysis of the stimuli at work in ontogenetic development, of the potentialities whose expression is conditioned by these stimuli, and of the cellular organization out of which the potentialities arise.

By means of such an analysis in terms of the individual cell, organic evolution must likewise ultimately be explained. Theories of the causes and of the course of evolution have been developed, in the main, in terms of the organism. All their sound elements can easily be restated in terms of the cell. Current discussions of evolutionary problems demonstrate that no material further progress is to be made by the use of the old terms and phrases. Consider, for example, the time and energy that are being wasted in controversies over the nature, the inheritance, and the modification of "congenital" and "acquired" characters, in which the characters are treated as qualities of the organism as a whole or of its constituent organs. There are no "characters" of an organ, still less of an organism, save in a figurative or abstract sense. What concretely exists and is inherited or modified from generation to generation is the fundamental constitution of the living matter, giving rise to certain potentialities and in some degree limiting and directing their expression. The organism is the resultant of the expression by each cell, under the conditions set by its constitution and by its environment, of some of its inherent potentialities.

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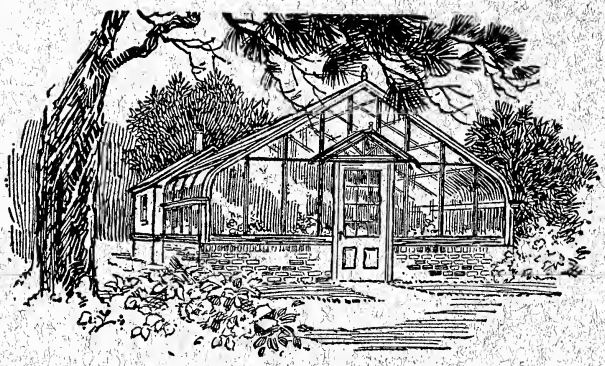

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THE EFFECT OF HEAT UPON THE MYCELIUM OF CERTAIN STRUCTURAL-TIMBER-DESTROYING FUNGI WITHIN WOOD [1]

WALTER H. SNELL

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The possible use of heat for checking decay in buildings has been suggested by various European writers, but little has been done either in an experimental or in a practical way to test its efficacy. Falck (1, p. 197; 2, pp. 338–340) made some tests upon the effect of heat upon the mycelium of some wood-destroying fungi in agar and in wood. The tests were not run in a systematic series, and Falck's only conclusion was that, whereas heat may be applicable for the checking of *Merulius lacrymans* within structures, it is not feasible in the case of *Lenzites sepiaria*, for heat is necessary for long periods and at high temperatures to kill this fungus. Hoxie (3, pp. 38–40) reports an experiment carried out in a mill infected with *Merulius lacrymans* and *Coniophora cerebella*, in which encouraging results were obtained by heating the building by means of its own heating system four times from Saturday noon to Monday morning to about 115° F. (46° C.).

The present paper gives the results of a series of tests upon the resistance to heat of the mycelium of certain wood-destroying fungi within wood and a discussion of the application of these results to the heat treatment of decayed timber in buildings, and to the kiln-drying and preservation of wood. The fungi are five found to be of importance in the decay of mill timbers, *viz.*, *Lenzites sepiaria*, *L. trabea*, *Trametes serialis*, *T. carnea*, and *Lentinus lepideus* (6).

METHODS

The cultures of the five fungi were made in 2-liter Erlenmeyer flasks in the manner described by Humphrey (5), using Sitka-spruce blocks $\frac{3}{4}$ by $\frac{3}{4}$ by 2 inches. Tests were made upon blocks from cultures incubated 4 months and from cultures incubated 1 year. In one case the blocks were thoroughly

[1] This work was suggested to the writer by Dr. C. J. Humphrey, begun at the Laboratory of Forest Pathology, Bureau of Plant Industry, Madison, Wis., and finished in the Botanical Laboratory at Brown University.

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invaded by the mycelium and a little decayed, and in the other they were badly decayed and partly dried. Blocks were tested against both dry and moist heat, the moist heat being obtained by supporting the blocks over water in a sealed Mason jar which was placed in the drying oven beside the blocks subjected to dry heat. The blocks to be tested with dry heat were removed from the flasks and air-dried in a warm room for several days, since otherwise, being wet inside, they would for a few hours in reality have been subjected to a moist heat. The blocks to be tested with moist heat were taken fresh from the culture flasks and tested in their partially moist condition. For the tests, the blocks were cut transversely in half, each yielding two pieces $\frac{3}{4}$ by $\frac{3}{4}$ by 1 inch in size. A sufficient number of blocks were placed in the oven at the beginning of the test, and one was removed at the end of each desired interval from 12 hours up to several days, varying with the temperature.

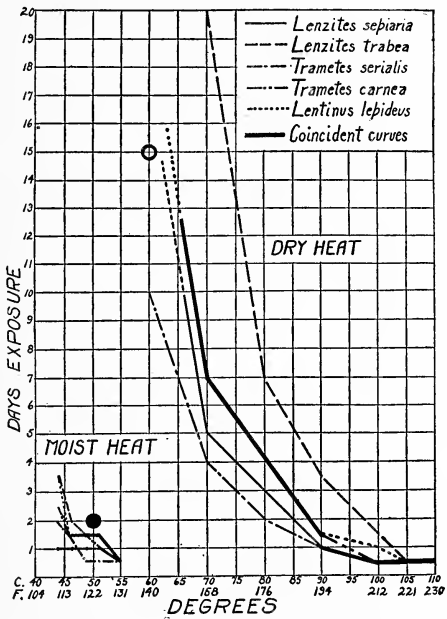
The viability of the mycelium within the block after being subjected to heat was tested by cultures made from the individual blocks. These cultures were made in a culture case kept swabbed with $HgCl_2$, with instruments dipped in alcohol and flamed, upon a block of wood similarly treated. The outer part of the block was flamed, and a slice was removed from each of the six sides. From the remaining inner part, ten pieces of wood material were put in as many tubes of malt agar and set aside to incubate. At least six weeks were allowed for the viable mycelium within the wood to grow out upon the agar.

Results [2]

The results are given in tables 1 and 2 and text figure 1. Inasmuch as little difference was observed between the behavior of mycelium from old and of that from new cultures, the differences are not compiled here. In a few instances, blocks from fresh cultures gave growth where those from old ones did not; in one case the situation was reversed, and in others there was no difference. It was to be expected that some irregularities would occur. For instance, *Lenzites sepiaria* produced no growth at 50° C. moist heat, but one tube of the ten in two tests at 52° C. showed growth. Again, at 52° C. moist heat, old mycelium of *Lentinus lepideus* showed growth after 36 hours' exposure in the first test, but two repetitions with old and one with fresh mycelium failed to show growth. In some tests there might be growth from the block exposed 48 hours and none from the one exposed 36 hours. In the case of the vigorous *Lenzites trabea* at 48° C. moist heat, one test gave no growth at all at 12 hours, while a repetition with blocks from the same culture showed that 24 hours' exposure did not kill the fungus. In the main, however, the results were reasonably uniform.

[2] Some preliminary results were presented in a paper read before the American Wood Preservers' Association (7). At that time it was pointed out that certain of the periods at the lower temperatures, with dry heat (60 percent and 70 percent more particularly), were thought to be incorrect and repetitions were to be made. These questioned tests have been repeated and the discrepancies between the curves in the two papers are thus explained.

In general, the resistance of the mycelium to dry heat was greater than to moist heat, but the degree of difference was somewhat surprising. Three and one half days' exposure to 44° C. and 12 hours at 55° C. moist heat was necessary to kill all the fungi, while with dry heat, the three-day killing period was from above 70° C. to above 90° C. and all the species were not killed in 12 hours until 105° C. was reached. Three of the fungi survived 12 hours at the boiling point of water in dry heat, and one of these (*Lenzites trabea*) survived 24 hours at that temperature.



TEXT FIG. 1. Graph showing the killing point of the mycelium of five "mill-roof" fungi within $\frac{3}{4}$- by $\frac{3}{4}$- by 1-inch spruce blocks, by both moist and dry heat. The open circle denotes that three of the fungi—*Lenzites sepiaria*, *Trametes carnea*, and *Lentinus lepideus*—were not killed at the point marked, which was the last test in that series. The closed circle denotes graphically the maximum amount of heat theoretically available in a mill over the week-end shut-down.

As was to be expected, there was individual variation in the susceptibility to heat of the fungi used. Further, it is apparent that the individual resistances of the mycelia of the different fungi to temperatures above those favorable for growth bear no relation to the temperature curves for growth (*cf.* 6). For example, *Lenzites sepiaria* is a high-temperature organism, having an optimum for growth upon agar between 32° and 35° C., and grows at 40° C., even at 44° C. according to Falck (1, pp. 127–129). The optima for the other fungi are around 28° C. and 30° C. None of the latter will grow at all at 40° C., and some not above 36° C. (6). Yet the mycelium of *L. sepiaria* was killed more easily than that of three of the other fungi and almost as easily as that of the least resistant of the group—*Trametes serialis*.

The mycelium of *Lenzites trabea* was decidedly the most resistant to heat, more especially dry heat. It was killed in 12 hours of exposure to 105° C., but survived one day at 100° C., 3 days at 90° C., 6 days at 80° C., and 19 days at 70° C. The greater resistance of the mycelium of this species was naturally attributed to the possible presence of some resistant spores formed within the wood, but repeated examinations of wood infected with pure cultures of this fungus have not yet revealed the presence of secondary spores, although both oidia and chlamydospores are present in cultures upon agar media. The mycelium of the least resistant of the five (*Trametes serialis*) succumbed after 10 days' exposure at 60° C., 4 at 70° C., 2 at 80° C., and 1 at 90° C. The thermal death points at dry heat for the other three fungi lie nearer to those of *Trametes serialis* than to those of *Lenzites trabea*.

With moist heat, irregularities were more common and several repetitions were necessary to gain an idea of the resistance of the various fungi. Both *Lenzites trabea* and *Lentinus lepideus* in most cases survived 3 days at 44° C., and for varying periods up to 52° C. *Trametes carnea* in part resisted 48° C. for 24 hours, and in one case 52° C. for 12 hours. In these latter tests, however, growth occurred in only 2 or 3 tubes. *Lenzites sepiaria* survived no more than 12 hours at any of the temperatures of moist heat tried, and in one of the two tests at 52° C., growth occurred in only 1 of 10 tubes.

The delaying effect of the more severe conditions, either of length of exposure or of degree of heat, in the growth of the mycelium from the test pieces on to the agar, was quite noticeable in four of the fungi, but less so in *Lenzites trabea*. This species responded to the test-tube conditions almost as well after periods of heat as in control tests. The growth in the tubes was slower in appearing, the more rigorous the exposure. Whereas a good culture may be obtained in 3 or 4 days from unheated wood such as used in these tests, growth from the more severely heated blocks was delayed until 3 weeks or 25 days for four of the fungi. If the mycelium in a block was ever to grow, it always appeared within a month.

Bearing of these Results upon Heat Treatment of Timbers in Buildings

The tests described in this paper were not made with the idea of obtaining practical results regarding the possibility of checking decay in buildings. They were intended simply as preliminary experiments and to be used as a guide in more extended work upon larger pieces of wood. However, even with the small blocks used, the results are so striking as to be worthy of discussion and useful in forming certain conclusions.

These results will apply very well to 1-inch stock, which is usually planed down to $\frac{7}{8}$ inch, because the difference in the time of heating the $\frac{7}{8}$-inch and $\frac{3}{4}$-inch material would be slight. It is quite certain that heat would act more slowly upon mycelium in large beams and planks than it would in the

$\frac{3}{4}$-inch blocks used in the tests. Hence, for example, if different degrees of heat which would be safe to use in buildings would not kill the mycelium in the small blocks, it certainly is hopeless to try heat to sterilize timbers in structures which are infested with the fungi considered here.

In this connection, there are a few things to be considered. One of these is that different wood-destroying fungi react differently to heat. *Merulius lacrymans*, the true dry-rot fungus (or what has thus far been taken for *M. lacrymans*), is a low-temperature organism and is more susceptible to treatment with heat than is the group of organisms considered here. According to Falck (1, p. 129), this fungus (which he calls *M. domesticus*) has an optimum around 21° C. and does not grow at all on agar above 27° C., a point lower than the optimum for any of the five fungi treated in this paper. While it has been pointed out above that there is necessarily no relation between the relative temperature curve and the relative resistance to heat above the growth temperatures (as in the case of *Lenzites sepiaria*), it is known that certain species of Merulius are more susceptible to higher degrees of heat, as would be expected from the growth relations of certain of the species studied. Hence, these tests do not apply to Merulius or to other fungi of the dry-rot group, but apply only to the species considered—those which are adapted to conditions found in cotton-mill weave-shed roofs.

Another consideration is that only dry heat and heat in a saturated atmosphere are considered here. What the effects of heat at the different humidities between the points of dryness and saturation would be can only be conjectured. The same heat applied in a dwelling and in a cotton-mill weave shed would obviously give quite different results because of the difference in relative humidity. The wetness of the wood would also need to be considered. In a dwelling house under ordinary conditions, the wood is below the fiber-saturation point. In a building where the air is saturated, the wood everywhere except in contact with the outside would be only at the fiber-saturation point, while that in contact with the outside would be above fiber saturation when it was cool outside. In one-story weave sheds, the moisture content of the roof planks is much above the fiber-saturation point, because the dew-point often comes within the plank and water is precipitated there. Hence, it is probable that heat applied in a room like a weave shop, even at humidities much below saturation, would act as the moist heat of a saturated atmosphere because of the moisture within the wood.

A further consideration with regard to the practical application of these results to mill-roof conditions is the impossibility of supplying the heat with sufficient uniformity. Although it might be deduced from the tables and graphs that a certain degree of heat for a certain period would kill the mycelium in stock of certain dimensions, it is of course apparent that heat applied in a structure such as a weave shed would be radiated so rapidly

that the available heat for sterilizing purposes throughout a roof plank, for instance, would be much less than that indicated as supplied.

From the practical point of view, the following conclusions may be drawn:

1. Dry heat in buildings in which the air is not artificially humidified is of no use for purposes of sterilizing wood infected with the five fungi discussed here. Too high a degree of heat for too long periods is necessary even in the small blocks to attain the end desired. A heating system in a building is capable of raising the temperature to only about 52° C. (125° F.), and such a temperature would not accomplish the sterilization. If automatic sprinklers are present, a temperature higher than 46° C. (115° F.) would not be safe.

2. Dry heat in dry structures applied for the purpose of drying out the wood and thus preventing or retarding decay is highly to be recommended. Much timber enters a structure in a partially green condition, and such timbers are especially susceptible to decay. A thorough drying before occupation would prevent decay such as often takes place when green or wet lumber is installed. A preliminary drying before painting timbers on the interior of buildings would be good insurance against decay. Green or wet timbers may be expected to decay rapidly in a moist atmosphere unless proper precautions are taken, but they often decay in dry buildings because they are painted over when wet inside. They then rot during the retarded drying and may become a total loss before sufficiently dried. For buildings which are already slowly decaying, a periodic application of dry heat as high and for as long a period as possible would dry the wood and retard the destruction.

3. Dry heat in structures of *moist occupancy* (really moist heat as explained above) would have to be applied as high as obtainable for periods longer than two days to accomplish anything. We speak of two days because that is the time during which heat might be applied in *operating structures*—from Saturday noon until Monday morning. From text figure 1, it is seen that temperatures from 44° to 50° C. (112° to 122° F.) killed the mycelium in the small blocks, but it is certain that not only would it take more heat for larger timbers, but also so much heat would be radiated that more would be necessary than is shown by the graph. Hence, the application of dry heat even in structures where the humidity is high cannot be recommended under ordinary conditions, and it is not certain that long periods of application would be of more value in eliminating these fungi.

4. Moist heat as such (humidified heat or free steam) need not be considered here. In a dry structure, it would be necessary to apply the moist heat for a period sufficient for the moisture to permeate the wood before the killing action took effect, and this would not be practicable under ordinary conditions. Secondly, dry heat applied to wet wood such as is found in weave sheds means that the fungi are in any case being subjected to moist heat within the wood.

Bearing of these Results upon Kiln-drying of Lumber and Structural Timber

The results obtained also throw some light on the effect of kiln-drying of lumber upon the mycelium of fungi within the wood. It is fair to assume that the results with these fungi will apply to the kiln-drying of coniferous timbers. The fungi under discussion in this paper cause most of the serious damage to worked timber, and hence the results obtained are directly

Table 1. *The effect of dry heat upon the mycelium of Lenzites sepiaria, L. trabea, Trametes serialis, T. carnea, and Lentinus lepideus within ¾- by ¾- by 1-inch Sitka-spruce blocks*[3]

		Temperature (degrees C.)						
	Days	60	70	80	90	100	105	110
Lenzites sepiaria	½	+	+	+	+	−		−
	1	+	+	+	−	−		−
	1½	+	+	+	−	−		−
	2	+	+	+	−			
	2½	+	+	+				
	3	+	+	−				
	4	+	+	−				
	5	+	−	−				
	6	+	−					
	7	+	−					
	15	+						
Lenzites trabea	½	+	+	+	+	+	−	−
	1	+	+	+	+	+	−	−
	1½	+	+	+	+	−	−	−
	2	+	+	+	++	−		
	2½	+	+	+	+++	−		
	3	+	+	+	+++			
	3½	+	+	+	−			
	4	+	+	+	−			
	5	+	+	+	−			
	7	+	+	−				
	8	+	+	−				
	9	+	+	−				
	12	+	+					
	19		+					
	20	+	−[4]					
Trametes serialis	½	+	+	+−	+−−	−	−	−
	1	+	+	−−	−	−	−	−
	1½	+	+	+−−	−	−	−	−
	2	+	+	−	−			
	2½	+	+					
	3	+	+					
	4	+	−					
	9	+						
	10	−						
	11	−						
	12	−						

[3] Each plus or minus sign refers to a single test of 10 tubes. A plus sign means that some growth was obtained, whether in one or all ten tubes, but in all but a very few cases it means growth in more than six of the 10 tubes of a single test.

[4] Test not satisfactory. Repetition may show growth.

TABLE 1.—(*Cont.*)

	Days	Temperature (degrees C.) 60	70	80	90	100	105	110
Trametes carnea.....	½	+	+	++	+−−	−	−	−
	1	+	+	++	+−−	−	−	−
	1½	+	+	++	−−	−	−	−
	2	+	+	++−−	−			
	2½	+	+	++−−	−			
	3	+	+	++−−				
	4	+	+	−				
	6	+	+	−				
	7	+	−					
	8	+	−					
	9	+	−					
	15	+						
Lentinus lepideus.....	½	+	+	++	++−	+	−	−
	1	+	+	++	+−−	−	−	−
	1½	+	+	++	−−	−	−	−
	2	+	+	+	−−−	−		
	2½	+	+	+	−			
	3	+	+	+				
	4	+	+	−				
	5	+	+					
	6	+	+					
	7	+	−					
	8	+	−					
	9	+	−					
	15	+						

applicable. If some of the rarer fungi attacking coniferous woods are more resistant to heat than the resistant *Lenzites trabea*, these results and the discussion following do not apply. Just how far they would hold for hardwoods which are infested with different fungi can only be conjectured, although *Lenzites trabea* is common on hardwoods and *L. sepiaria* occasionally occurs on them. In the remainder of the paper, however, it is assumed that by far the greater part of the combat against structural-timber-destroying fungi is against species to which these results are applicable, *i.e.*, the five considered here and others like Merulius which are less resistant to heat. In applying these results to kiln-drying, it is assumed that the effect upon 1-inch stock would be about the same as upon the ¾-inch blocks used in the experiments. In processes in which the kilns are run at a fairly uniform temperature and humidity, the possible killing effect of the heat used may be determined from the curves presented (text fig. 1). In the case of softwoods dried directly from the saw in a green condition with high temperatures, any mycelium should be killed, inasmuch as none of the fungi used, which are the commoner ones, could stand 12 hours at 55° C. moist heat. In the case of hardwoods, the killing of wood-destroying mycelium would depend upon the combination of time and temperature (if not the species of fungus)—especially, perhaps, upon the

TABLE 2. *The effect of moist heat upon the mycelium of Lenzites sepiaria, L. trabea, Trametes serialis, T. carnea, and Lentinus lepideus within ¾- by ¾- by 1-inch Sitka-spruce blocks*

		Temperature (degrees C.)					
	Hours	44	46	48	50	52	55
Lenzites sepiaria	12	+	+	−−	−	++	−
	24	−	−	−	−	−	−
	36	−	−				
	48	−	−				
	60		−				
	72		−				
Lenzites trabea	12	+	+	+−	+	+−	−
	24	+	++	+−	−	+−	−
	36	+	+	−		−	−
	48	+	−	−		−	−
	60	+	−				
	72	+	−				
Lenzites serialis	12	+−	++−	−−−−	−	−−	−−
	24	+	+++	−−−	−	−	
	36	+	−−	−−			
	48	−	−−				
	60	−					
	72	−					
Trametes carnea	12	+	+	+−	−	+−−	−−
	24	+	+	+−	−	−	
	36	+−	−	−−−			
	48	+	−	−−			
	60	−	−	−			
	72	−	−	−			
Lentinus lepideus	12	+	++	+++	+	+++	−
	24	+	+−	−	−	++−	−
	36	+	−			+−−−	−
	48	+−	−			−−−	−
	60	+	−			−−	−
	72	+−	−			−−	−

time during which the moisture content of the wood was above the fiber-saturation point. It may be seen that it would not take very long periods at temperatures from 45° C. upward, with the wood at fiber saturation (moist heat), to kill all mycelium. Green hardwoods are usually under these conditions long enough to be sterilized within. Preliminary steaming treatments would have the same effect.

In the controlled-humidity type of kiln, the same should hold true. An analysis was made of the curves given by Tiemann (8, Pls. I–VII) which show the conditions operating upon different kinds of 1-inch lumber in a typical run of this type of kiln (table 3). For our purposes here and for comparison with the data presented in this paper, the possibility of killing mycelium in wood in such a kiln may be calculated in two ways: (1) on the basis of dry heat acting over the entire period of the run at the varying temperatures, and (2) on the basis of moist heat acting from the beginning of

TABLE 3. *Data taken from Tiemann's curves of temperature and humidity in characteristic dry-kiln runs (8, pp. 277–285) for comparison with data in text figure 1 to show probable effect of kiln-drying on wood-destroying fungi within the wood*

DRY HEAT [5]

Curve no.	Kind of Wood	Entire Run of Kiln		*Probable* Killing Effect as Taken from Curve in Text Figure 1.
		Temperature (C.)	No. of Days	
1	1-inch hardwood	50–65°	20	None of the fungi would be killed.
2	1-inch hardwood	50–70°	14	None of the fungi would be killed.
3	1-inch hardwood	77–80°	7	None of the fungi would be killed.
4	2½-inch hardwood	43°	64	None of the fungi would be killed.
5	1-inch conifer	75°	10	All but *Lenzites trabea* would probably be killed.
6	1-inch conifer	57–77°	10	No killing effect.
7	1-inch conifer	82–93°	4	*L. trabea* would not be killed, others might be.

MOIST HEAT

Wood at Fiber-saturation Point

1	1-inch hardwood	50°	6	2 days at this temperature would probably kill all fungi.
2	1-inch hardwood	50–55°	8	2 days at this temperature would probably kill all fungi.
3	1-inch hardwood	75° at least	3	12 hours at 55° kills all fungi.
4	2½-inch hardwood	45° at least	25	4 days kills all in ¾-inch material; 25 days should kill all mycelium in 2½-inch stock.
5	1-inch conifer	75°	2–3	2 days at 50° kills all fungi.
6	1-inch conifer	57°	2–5	All killed in 12 hours at 57°.
7	1-inch conifer	82–88°	1½	1½ days at 52° kills all fungi.

the run until the time when the water content of the wood goes below the fiber-saturation point. In the second contingency, the air within the wood is saturated with moisture, and hence the heat applied acts as moist heat regardless of the external humidity, which is, however, high for most of the period.

Taking the results upon five fungi, it appears from the two sets of data (text fig. 1 and table 3) that such a procedure may be counted upon to kill the mycelium of most if not all species in 1-inch stock. Counting the heat applied in these kilns as absolutely dry heat over the whole period (which it certainly is not), it is seen that in most of the cases the mycelium would not be killed. On the other hand, it is seen that moist heat is acting upon the mycelium in the wood in all of these seven conditions (table 3) sufficiently

[5] It is of course understood that the heat for the entire run of the kiln cannot be dry. It is considered as dry for the purposes of this treatment, solely for comparison with the data on dry heat presented and because there are no data available for humidities between dryness and saturation of the air. See text for discussion.

long to kill it. The effect of the same temperatures at intermediate humidities can not be determined.

These results are taken to apply only to 1-inch stock, but with longer periods of treatment of larger material the results should be the same. Moist wood is a fairly good conductor of heat. Furthermore, there is in most cases a sufficient margin between the time necessary to kill the mycelium in 1-inch stock and the time that even the 1-inch lumber is exposed to killing temperatures to allow heat to penetrate to the center of large material and to act upon any mycelium. Most of the decay in structural timbers without doubt owes its incipiency to infection between the time of cutting and the time of installation. These results indicate that kiln-drying could be counted upon to render structural timbers sterile internally as far as most if not all wood-destroying fungous mycelium is concerned, and that in kiln-drying we have an important agent in combating decay in buildings.

Bearing of the Results upon the Possible Sterilizing Effect of Wood-preservation Processes

The results given in this paper indicate also that various wood-preservation processes should be sterilizing processes as well. The necessity for sterilizing treated wood is not everywhere understood. It is often noted that treated wood decays inside in the untreated portion leaving a hollow shell which has been protected. This decay within may have started in two ways. The fungi may have entered through checks or nail holes, etc., *i.e.*, through breaks in the protecting preserved layer, or it may have arisen from fungous mycelium which entered the wood between the time of felling of the tree and the time of preservative treatment. It is certain that a great deal of decay in structural timber owes its origin to this method of infection, as already pointed out. Hence, it is important that the inside of the treated timbers should be sterilized, as otherwise the preservative treatment may be a waste of time and money. This discussion, of course, advocates the treatment of wood already infected only in the sense that all timber apparently sound may be and in a great many cases probably is infected with fungous mycelium before it is installed, and refers only to those preservative treatments in which a fairly high degree of heat is applied. This is very different from even suggesting any kind of preservative treatment, with or without heat, for wood already visibly infected or partially decayed.

Wherever heat is applied, either in a preliminary seasoning treatment or in the preserving process itself, it is possible that the heat will be sufficient to kill any fungi within the wood. It may make some difference whether or not the wood is green or partially dry. If the wood is green, or is wet enough so that the air in the wood cells will be saturated, the heat applied will react as moist heat, and the amount of heat necessary for sterilization purposes under these conditions is not great, since 55° C. for 12 hours will

kill all the fungi tested in $\frac{3}{4}$-inch stock. If the wood is air-dry, it is possible that there will be sufficient moisture in the wood to volatilize under the heat treatment and thus to cause the heat applied to act as moist heat; or the liquid applied may penetrate and produce the same result. Further, of course, it is always possible that the heat may be applied long enough to accomplish the sterilization, even as dry heat.

In such preliminary treatments as applying saturated steam, superheated steam, or hot oil, there should be sufficient heat present to kill all mycelium in wood, especially if aided by subsequent vacuum treatments to rupture the fungous cells. In many preservative processes, the same should be true. For instance, in a treatment such as described by Hoxie (4) for treating material for insulation on a mill roof, the $\frac{7}{8}$-inch pine sap boards were immersed in creosote at a temperature of about 105° C. (220° F.) for 20 hours or more—long enough to accomplish the desired sterilization even on the basis of dry heat, in addition to protecting the wood against future attack. In several pressure processes, the preservative is heated up to 93° C. (200° F.) or above, and the heat is probably high enough for a long enough period to sterilize the wood in many, if not all, cases even on the basis of dry heat.

Summary

Inasmuch as the application of heat to various structures has been suggested as a possible remedy against decay, five fungi found growing in cotton-mill roofs were tested as to their thermal death relations in moist and dry heat. Species of Merulius and other fungi of the dry-rot group are not considered here.

The tests were made upon blocks of Sitka spruce $\frac{3}{4}$ by $\frac{3}{4}$ by 1 inch taken from 4-months- and 1-year-old cultures of the five fungi used and subjected to both moist and dry heat for varying intervals and at varying temperatures.

In moist heat, the most resistant of the fungi was killed in $3\frac{1}{2}$ days at 44° C. and in 12 hours at 55° C. In dry heat, 20 days at 70° C. did not kill the most resistant, nor did 12 hours at 100° C., although all succumbed in 12 hours at 105° C. dry heat.

There were individual differences in the resistance of the various fungi, and the individual curves bore no direct relation to the thermal growth curves. *Lenzites sepiaria* has the highest optimum and maximum of growth of the fungi tested, but next to the lowest thermal death curve. *Lenzites trabea* proves to be by far the most resistant of the five fungi, although its thermal growth relations are about the same as those of the other three fungi.

It is concluded even from the results upon the small blocks that heat applied to buildings as a sterilizing agent can be of little avail against the five fungi tested, although it is pointed out that periodic heatings of such

structures might be of service in checking decay through drying out of the timbers. Heating before structures are painted or occupied is recommended.

Inasmuch as the five fungi tested are the most common destroyers of structural timber and are more resistant to heat than the dry-rot fungi (*Merulius* spp. and others), it is concluded that various kiln-drying and wood-preservation processes should sterilize the wood treated, inasmuch as the data show that sufficient heat is applied in most, if not all, cases to accomplish this result.

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AN ISOELECTRIC POINT FOR PLANT TISSUE AND ITS SIGNIFICANCE[1]

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The study of the relation of hydrogen-ion concentration to the growth and development of plants or plant parts has, in many cases, indicated that there is between the extreme limits of acidity or of alkalinity which are injurious to and interfere with growth and development a third region where the reaction affects the plant injuriously. This produces, when the growth of the plant or the intensity of the process is plotted against the hydrogen-ion concentration of the solution, a curve having a double maximum with a minimum located between the two maxima. Webb (25) was apparently the first to call attention to this phenomenon. He found that, when the spores of *Penicillium cyclopium* were germinated in buffer mixtures of H_3PO_4 and NaOH containing mannite, two maxima appeared with a minimum between when the percentage of spore germination was plotted against the pH of the solutions. He found similar curves for *Fusarium* sp., and, under certain temperature conditions, for *Aspergillus niger*. For *Botrytis cinerea* and *Lenzites saepiaria* his curves showed but one maximum. In a more recent paper (26) he has studied the germination of the spores of a large number of fungi in various media the hydrogen-ion concentration of which was adjusted by means of H_3PO_4 and NaOH. In various forms, notably Penicillium and Fusarium, and under certain conditions, two maxima were found in the curve of spore germination plotted against the hydrogen-ion concentration of the medium. In at least one set of conditions, two maxima were found for *Aspergillus niger*, *Penicillium cyclopium*, *Botrytis cinerea*, *Fusarium* sp., *Penicillium italicum*, *Lenzites saepiaria*, and *Puccinia graminis*. Only one maximum was found for *Colletotrichum Gossypii*.

Salter and McIlvaine (22) found a double-maximum curve for the growth of wheat seedlings in solution cultures the reaction of which was adjusted with citric acid and NaOH. They concluded that this curve resulted from the exhaustion of nitrates due to the development of bacteria in the cultures.

[1] Published with the permission of the Director of the Agricultural Experiment Station, University of Missouri. The experiments on the absorption of water by potato-tuber tissue in buffer mixtures of H_3PO_4 and NaOH were performed for the writer by Mrs. Edward Abbott. The assistance of Mr. Karl Petsch in the experimental work involved is also acknowledged.

Hixon (9) investigated the germination and seedling growth of peas, corn, wheat, oats, and carrot in Tollen's solution, in agar made from tap water, in tap water, or in Tollen's solution made up in tap water. In each case the reaction of the medium was adjusted with HCl or NaOH to a pH of approximately 3.0, 4.0, 5.0, 6.0, and 7.0. He found a double-maximum curve for the germination and seedling growth in length of peas with the minimum at pH 5.0. A double-maximum curve was found for the germination and seedling growth in length of corn, wheat, and oats with the minimum at a pH of 6.0 between the two maxima. For the germination of carrots a double-maximum curve with minimum at pH 5.5 was found, although the curve for the seedling growth showed but one maximum. The wet weight and dry weight of the roots of wheat seedlings grown for 16 days showed a minimum at pH 5.25 with increase in weight on either side of the minimum as the alkalinity or acidity increased, the increase being greater on the alkaline side. The ash content was less in the solutions more acid than pH 6.0 and greater in solutions more alkaline than pH 6.0. The growth of the plants tended to shift the reaction of the more acid solutions toward greater alkalinity and that of the more alkaline solutions toward greater acidity. Hixon quotes a suggestion by E. J. Cohn that the minimum point is the isoelectric point of a protein of the cell membrane of the seed.

Olsen (18), according to Arrhenius, found that some plants showed a double-topped curve when the growth was plotted against the pH of the medium. He explained this as due to the presence of two different strains of plants.

Hopkins (11) in this laboratory grew *Gibberella saubinetii* in liquid media the reaction of which was adjusted by means of H_2SO_4 and NaOH or the potassium phosphates, H_3PO_4 and KOH, and on potato-dextrose agar the reaction of which was adjusted by means of lactic acid. He found that the growth showed a double-maximum curve when plotted against the hydrogen-ion concentration of the medium. The minimum for this fungus was found at pH 5.5–6.0. In soil cultures in which the reaction was adjusted by H_2SO_4 and NaOH, or by HCl and NaOH, he also found a double-maximum curve when the rate of germination of wheat or the seedling infection of wheat by *Gibberella saubinetii* was plotted against the hydrogen-ion concentration of the soil. The minimum for the number of wheat seedlings up in 4 days was at pH 5.4–5.6, and for infection at pH 5.2–5.5. The conidial germination of *Gibberella saubinetii* (10) was also found to show a double maximum when the percentage of germination was plotted against the hydrogen-ion concentration of the solution used as a medium for germination.

Cole (5) found that the increase in length of seedling corn roots in a 2–4 day period in buffer mixtures of H_3PO_4 and NaOH, or KH phthalate and NaOH, showed a double-maximum curve when plotted against the pH of the solution. The minimum was located at about pH 6.0.

Arrhenius (1) calls attention to the double-maximum curves secured by Hixon and Salter and McIlvaine and states that the same type of curve is also secured when the growth of older plants is plotted against the pH. He cites data for $2\frac{1}{2}$-months-old plants in soil cultures showing that the relative leaf area of bersim (*Trifolium alexandrium*), barley, corn, and cotton showed curves with double maxima. The minimum between the two maxima for bersim was found at pH 6.0, for barley at pH 8, for corn at pH 5 and pH 7, and for cotton at pH 8. In solution cultures $2\frac{1}{2}$ months old, wheat plants and radish plants showed a double-maximum curve for the weight of roots plotted against the pH, the minimum between the two maxima falling at pH 6.0. The weight of stems of wheat plotted against the pH of the solution also showed a double-maximum curve with a minimum at pH 6.0.

The cause of this double maximum and minimum is suggested by Arrhenius as being due to changed intensity of permeability for the different salt nutrients, or to the fact that the solubility of salts differs at different hydrogen-ion concentrations.

Although attention was directed by Webb, Hixon, Hopkins, and Arrhenius to the breaks or minimum points in the curve of the germination of spores and seeds, of the growth of plants or of infection of host plants by fungi when the intensity of the process is plotted against the pH of the medium in which the plant material is placed, similar observations can be found in the older literature. In these cases the hydrogen-ion concentration was not measured, and the results are therefore less certain in their interpretation.

Fischer (8) found that treatment with dilute acids or with solutions of acid salts markedly increased the germination of seeds of *Sagittaria sagittaria* or *Sagittaria platyphylla*, which showed almost no germination in distilled water or in solutions of neutral salts such as NaCl or $CaCl_2$. Dilutions of KOH or NaOH had the same favorable effect as the acids. Copper sulfate also increased the percentage of germination.

Dachnowski (6) found that in dilute acids, $\frac{N}{800}$, and in dilute alkalies, $\frac{N}{800}$, bean and corn seeds showed a greater maximum water absorption than in distilled water.

F. E. Lloyd (12) studied the growth rates and accompanying phenomena in pollen tubes of *Phaseolus odoratus*. Increasing the concentration of acetic, malic, or citric acid in 40 percent cane sugar increased the rate of growth of the pollen tubes to a maximum at $\frac{N}{3200}$, $\frac{N}{12800}$, or $\frac{N}{12800}$ respectively. Further increase in the concentration of the acid decreased the growth rate. Increasing the amount of NaOH in 20 percent cane sugar also increased the growth rate to a maximum at $\frac{N}{3200}$. Further increase in

the concentration of NaOH inhibited the growth. Although the hydrogen-ion concentration was not measured, it would appear that the growth of pollen tubes of *Phaseolus odoratus* in solutions of varying hydrogen-ion concentration should produce a double-maximum curve if plotted against the pH of the solutions.

Cohen and Clark (4) figure a double-maximum curve for the number of viable cells of *B. dysenteriae* (Shiga) at the end of 5.5 hours in media of different hydrogen-ion concentrations. Hopkins (11) has indicated that the curve given by Brightman, Meecham, and Acree (2) for the growth of *Endothia parasitica* at various hydrogen-ion concentrations may be interpreted as having a double maximum.

Although there is an increasing body of evidence to indicate that the occurrence of a double-maximum curve is to be expected when the influence of hydrogen-ion concentrations upon various physiological functions or processes of plants is investigated, a considerable amount of work has been reported which shows no such phenomenon. This should not be considered as necessarily indicating the non-existence of a double-maximum curve. Many conditions may conceivably conceal or obliterate the minimum located between the two maxima. Thus the pH values of the solutions may be separated so far that the minimum is not found. Growth conditions such as temperature, water supply, container size, or salt content of the medium may so limit the development that the values of the two maxima may lie close to the minimum value and be indistinguishable from it.

A minimum located between two maxima also occurs when the swelling, the osmotic pressure, the viscosity, the electrical conductivity, and the alcohol number of gelatin in solutions of varying reaction is plotted against the reaction of the solution expressed as pH. This minimum is located at the isoelectric point of gelatin, pH 4.7. The possibility that there might be an isoelectric point for living tissue and that the existence of an isoelectric point might account for the double-maximum curves and the minimum to which attention has been called in the discussion given above has led to the investigation which is reported in this paper.

The Isoelectric Point

Before describing the experiments involved in this investigation, a brief statement of the meaning of the isoelectric point will add to the clearness of the later discussion.

The conception of an isoelectric point is due to Hardy, who found that the direction of movement of a protein in an electric field is determined by the reaction of the fluid in which it is suspended. Since a hydrosol in which the particles are electro-positive can be changed by the addition of free acid until the particles become electro-negative, it is evident that there must be some point at which the particles and the fluid in which they are immersed are isoelectric. Michaelis and Mostynski (15) defined the isoelectric point

of a protein as that reaction where the relation of the concentration of the hydrogen ions to the hydroxyl ions in the solution is the same as the relation of the acid-dissociation constant (Ka) of the protein to its basic-dissociation constant (Kb). At the isoelectric point the sum of the number of protein anions is equal to the number of protein cations present, and the sum of the protein ions in relation to the non-ionized protein is at its minimum.

The significance of the isoelectric point for the physical and chemical properties of proteins has been pointed out by Hardy, Procter and Wilson, Loeb (13), and others. At the isoelectric point the physical properties of such a protein as gelatin are at a minimum. Thus, if the swelling of the same quantity of gelatin in solutions of different hydrogen-ion concentrations is measured, it is found that the swelling is least at the isoelectric point, pH 4.7. In solutions of greater acidity or alkalinity than this, the swelling increases to a maximum. The same is true of the viscosity, osmotic pressure, conductivity, and alcohol number of gelatin, as has been pointed out by Loeb. A protein can combine with anions only on the acid side of the isoelectric point and with cations only on the alkaline side of the isoelectric point. In other words, a protein may act as either a base or an acid, the reaction of the solution with the isoelectric point as the critical one determining which it shall be. From the above discussion it is evident that there are available several methods of determining the isoelectric point of a protein. It can be determined by the method of Hardy, *i.e.*, by observing the migration of particles in an electric field in solutions of different pH. It can be determined by measuring the swelling, viscosity, osmotic pressure, or electrical conductivity of the protein. It can also be determined by determining in what H-ion concentrations cations or at what hydrogen-ion concentrations anions do and do not combine with the protein. These methods have largely been developed by Loeb (13) chiefly by a study of gelatin.

Experimental

The experimental work described in this paper includes experiments which deal with the absorption of water by potato-tuber tissue in solutions of different hydrogen-ion concentrations and experiments on the absorption of dyes by plant tissue which had been in contact with solutions of different hydrogen-ion concentration.

Experiments on Water Absorption

Potato-tuber tissue was used for the experiments on water absorption because it appeared to furnish the most easily available supply of fairly uniform material. One experiment was also carried out with beet tissue, but the material showed such great variation, probably due to the fact that stored beets, somewhat wilted, were used, that no conclusions could be drawn from the data and the results are not reported.

In the preparation of the tissue the general methods of Stiles and Jorgensen (23) were followed. By means of a cork borer, cylinders of the potato tissue were cut 1.5 cm. in diameter. These cylinders were then sliced into circles about 1 mm. in thickness. At first the slices were cut by hand with a razor. Later a slaw-cutter was used with part of the board cut away under the knife to permit the slices to cut cleanly. The slaw cutter produced pieces of uniform thickness and was much more rapid than the razor. After being cut, the potato slices were rapidly washed with distilled water to remove the starch set free in the cut cells and then blotted dry with filter paper until no evidence of free water was visible on the surface of the slices. After thorough mixing, they were weighed out in lots of 10 g. and dropped into 150-cc. quantities of the solutions used, contained in 150-cc. beakers of Pyrex glass.

Buffer mixtures of phosphoric acid and sodium hydroxide, of secondary sodium citrate and sodium hydroxide, or of potassium hydrogen phthalate and sodium hydroxide were used to maintain the desired hydrogen-ion concentrations. The disadvantages and advantages of using buffer mixtures instead of acids or alkalies only for studying the effect of hydrogen-ion concentration are well appreciated and need not be discussed at length here. The presence of ions other than the hydrogen and hydroxyl ions at concentrations which are necessarily not constant and which are far above those of the hydrogen and hydroxyl ions is unavoidable, and must be taken into consideration in interpreting the data. The buffer mixtures afford a means, however, of maintaining a definite hydrogen-ion concentration which could not be done if an acid or alkali alone were used.

After the potato tissue had stood in the buffer mixtures for 6 to 12 hours, the slices were removed, carefully blotted dry with filter paper, weighed, and returned to a fresh quantity of the same buffer mixture. After 12 or 24 hours, and again at the end of 24 or 48 hours, the process was repeated. In this way three sets of weighings at different intervals of time were made on each lot of potato.

Each treatment was performed in triplicate, and the average of the triplicate weighings is given in the tables or used in constructing the curves which follow. The hydrogen-ion concentration of the solutions in contact with the potato was also determined by Gillespie's colorimetric method each time the potato was weighed in order to learn how much the presence of the potato had affected the reaction. The accuracy with which the colorimetric method of determining the hydrogen-ion concentration was used probably did not exceed 0.1 pH. Difficulty was experienced with brom-cresol-purple. This indicator seemed to yield results which were about 0.2 pH lower than those secured with methyl red or brom-thymol-blue. Wherever possible, determinations were checked with a second overlapping indicator, but in the lower range of brom-cresol-purple this was impossible. All experiments were carried out at room temperature. This varied from

24 to 28° C., but was fairly constant for a given set and was of course uniform in its effect since one set was completed at the same time.

The amount of water absorbed or lost by the potato tissue was assumed to equal the difference between the original weight and that found at the given interval. This is not strictly correct. The absorption of salts from the solution or the loss of salts or organic matter from the potato will affect the weight of the potato. It is believed, however, that the amount of such exchange plays but a small part in the changes in weight which the potato tissue shows in these experiments. Ash determinations made in some of the experiments indicate that fact.

A second source of error, and one which can not be overcome, is the relation of the hydrogen-ion concentration of the interior of the potato cells to that of the solution by which they are bathed. Are they the same or different, and how long does it take for equilibrium to be reached? It is very probable that the H-ion concentration of the interior of the cells is not the same as that of the solution in which they are immersed. Because of the Donnan equilibrium, as has been pointed out by Loeb (13), the H-ion concentration of gelatin chloride in a collodion sac immersed in HCl is less than that of the HCl which surrounds the sac. We should, therefore, expect that the pH of the interior of the potato cells would not be the same as that of the outer solution when equilibrium is reached. No attempt has been made to discover the rate at which the H and OH ions penetrate the potato cells. However, it is probable that they enter rapidly if we can judge from the rapidity with which the color of the cell sap changes when pigmented cells are mounted in dilute acid or alkali. The use of discs about a millimeter in thickness avoided a mass of tissue in the center which would be slowly affected and assisted rapid equilibrium.

Phosphoric Acid—Sodium Hydroxide Series

The buffer mixtures in this series were prepared by mixing 0.1 M H_3PO_4 and 0.1 M NaOH in the proportions indicated by the titration curve for phosphoric acid and potassium hydroxide given by Clark (3). One experiment was performed using the concentrations secured by the use of the 0.1 M H_3PO_4 and NaOH. A second experiment was performed in which the original mixtures were diluted ten times with distilled water, and a third experiment was completed in which the diluted buffer mixtures were used but the range of hydrogen-ion concentration covered included only the region of pH 5.4–6.6. In the first two experiments the distilled water used contained calcium salts carried over in distillation sufficient to raise the pH to 9.0. In the third experiment water redistilled from acid and alkaline potassium permanganate was used in preparing the solutions. The calculated concentrations of H_3PO_4 and NaOH in mols per liter present in each of the buffer mixtures used in the first experiment are given in table 1. The concentrations given there represent those in the final mixtures. Thus,

in solution 3, equal parts of 0.1 M H_3PO_4 and 0.1 M NaOH were mixed. The final buffer mixture contained, therefore, if the two constituents which compose it are considered individually, 0.05 M H_3PO_4 and 0.05 M NaOH. It is recognized, of course, that the H_3PO_4 and NaOH do not exist as such in the buffer mixtures, but for convenience they are expressed as individuals. In the second experiment the concentrations were one tenth of those in experiment one.

In the first experiment of this series the potato was weighed at the end of 8, 12, and 24 hours. The amount of potato used for the solutions of different hydrogen-ion concentration varied from 10.06 g. to 10.16 g., but the gains or losses in weight were calculated per 10 g. of original potato tissue. An examination of the data showed that little change took place in the pH of the solutions as a result of standing in contact with the potato. There was a slight change toward greater alkalinity at the acid end. The increase in weight for the potato in the two most acid and the two most alkaline solutions was greatest at the end of 8 hours. For the balance of the solutions little change took place between 8 and 12 hours. By the end of 24 hours the potato in all solutions had begun to decrease in weight below the maximum. This loss was greatest in solutions 1, pH 2.0, and 3, pH 4.1, amounting to more than 0.9 g., and least in solution 5, pH 5.4, where it amounted to but

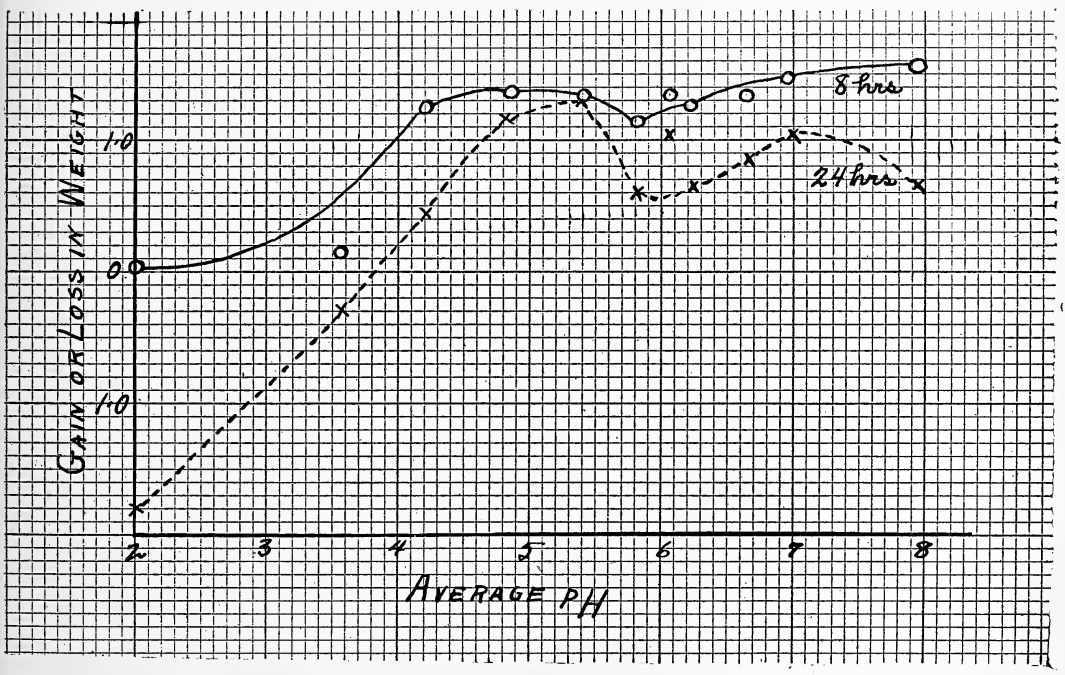

Fig. 1. The gain or loss in weight of 10 g. of potato-tuber tissue in 8 and 24 hours in 150-cc. quantities of buffer mixtures of 0.1 M phosphoric acid and 0.1 M sodium hydroxide. The buffer mixtures changed at 8, 12, and 24 hours.

0.09 g. It is noteworthy that in this solution the weight of the potato remained almost constant at all three weighings. In the other buffer mixtures the loss in weight varied from 0.29 g. to 0.66 g. with the majority around 0.50 g.

If the gain or loss in weight is plotted against the average hydrogen-ion concentration of the solutions expressed as pH, the curves given in figure 1 are obtained. The results secured at the end of 8 and 24 hours are given there. The curve for the 12-hour period is very similar to that for 8 hours, and to avoid confusion is not included in the figure. An examination of figure 1 shows that a smooth curve drawn through the points forms a curve in each case with a double maximum, a minimum occurring between at a pH of 5.8 to 6. If all points were connected, a W-shaped curve would result around pH 6.0 with a peak at pH 6.05. This has not been done in the figure. A consideration of the probable error showed that the minimum at pH 5.8–6.0 could not be accounted for by variation.

In order to reduce the effect of the ions other than H and OH present in the buffer mixtures, the solutions described above were diluted 10 times for the second experiment. The results are given in table 2. There it can be noted that, with less buffer action due to the dilution, the potato produced a greater change in the reaction toward alkalinity at the acid end of the series than was noted with the more concentrated mixture. A change in

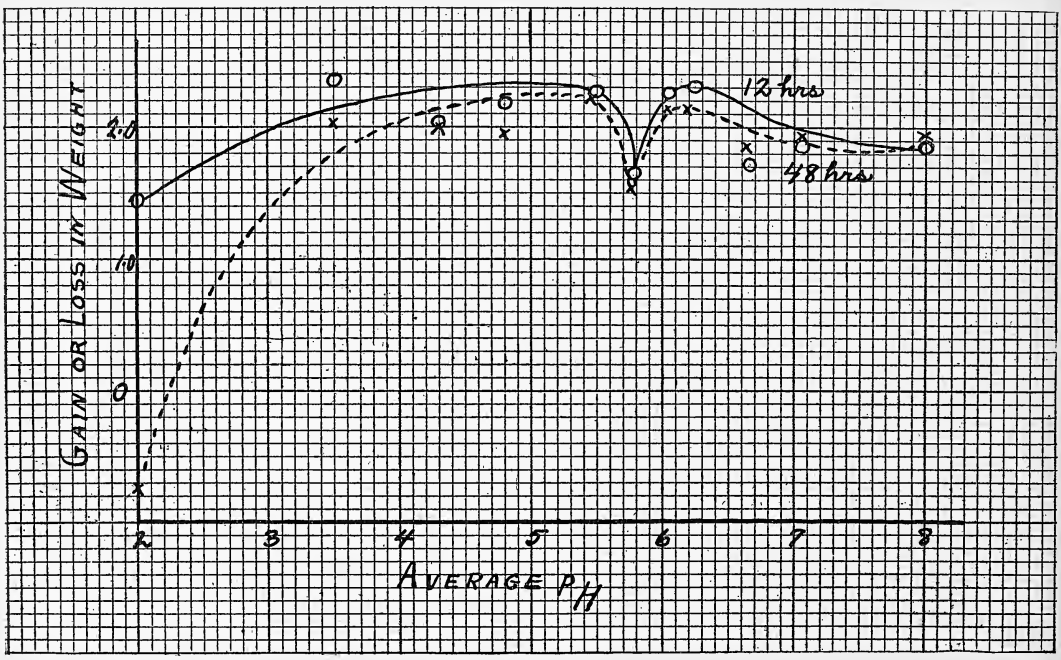

Fig. 2. Gain or loss in weight of 10 g. of potato-tuber tissue in 12 and 48 hours in 150-cc. quantities of buffer mixtures of 0.01 M phosphoric acid and 0.01 M sodium hydroxide. The buffer mixtures replaced at 12, 24, and 48 hours. See table 2.

reaction is not noted to the same extent or regularity in buffer mixtures of pH 5.8 and above. The increase in weight in this series was decidedly greater than with the stronger salts used in the first experiment. There was also a small continued increase at the end of 24 hours instead of the loss noted before. At the end of 48 hours, however, the potato in all solutions showed a decrease in weight below the maximum attained. This loss was again greatest at the acid end and least in the solution of pH 5.4, being but 0.03 g. there, while it was from 0.11 to 0.68 g. in the other buffer mixtures. The potato tissue in this solution again remained almost constant in its gain at the 12-, 24-, and 48-hour weighings.

If we plot the gain or loss in weight against the average pH of the solutions and draw a smooth curve through the points secured, as has been

TABLE 1. *The molecular concentration of the two constituents of the buffer mixtures used in experiments on water absorption*

Sol. No.	Phthalate		Citrate I		Citrate II		Phosphoric Acid	
	KH Phthalate	NaOH	Na Citrate	NaOH	Na Citrate	NaOH	H_3PO_4	NaOH
	mol.	mol.	mol.	mol.	mol.	mol.	mol.	mol.
1	0.01	0.00008	0.00980	0.00020	0.00200	0.00000	0.0714	0.0285
2	0.01	0.00150	0.00950	0.00050	0.00196	0.00004	0.0510	0.0488
3	0.01	0.00354	0.00900	0.00100	0.00180	0.00020	0.0500	0.0500
4	0.01	0.00599	0.00800	0.00200	0.00160	0.00040	0.0495	0.0504
5	0.01	0.00797	0.00700	0.00300	0.00140	0.00060	0.0490	0.0509
6	0.01	0.00860	0.00600	0.00400	0.00120	0.00080	0.0481	0.0519
7	0.01	0.00870	0.00575	0.00425	0.00115	0.00085	0.0471	0.0528
8	0.01	0.00890	0.00550	0.00450	0.00110	0.00090	0.0454	0.0545
9	0.01	0.00920	0.00525	0.00475	0.00105	0.00095	0.0417	0.0583
10	0.01	0.00940	0.00510	0.00490	0.00102	0.00098	0.0384	0.0615
11							0.0333	0.0666

TABLE 2. *The gain in weight of 10 g. of potato-tuber tissue in 12, 24, and 48 hours in 150-cc. quantities of buffer mixtures of 0.01 M H_3PO_4 and 0.01 M NaOH, the buffer mixtures being replaced with a fresh supply at each weighing*

Sol. No.	Initial pH	pH at End of 12 Hrs.	pH at End of 24 Hrs.	pH at End of 48 Hrs.	Gain or Loss, 12 Hrs.	Gain or Loss, 24 Hrs.	Gain or Loss, 48 Hrs.
					g.	g.	g.
1	2.0	2.0	2.0	2.0	1.41±.036	0.14±.02	−0.72±.06
2	3.5	3.5	3.5	3.5	2.33±.046	2.39±.06	2.05±.02
3	4.1	4.5	4.5	4.3	2.02±.08	2.36±.09	2.01±.02
4	4.7	4.9	4.8	4.9	2.18±.036	2.21±.01	1.94±.04
5	5.4	5.6	5.5	5.6	2.25±.01	2.31±.03	2.29±.01
6	5.8	5.7	5.75	5.8	1.66±.07	1.88±.05	1.51±.009
7	6.0	6.1	6.1	6.1	2.25±.04	2.38±.01	2.10±.04
8	6.2	6.2	6.4	6.2	2.30±.02	2.31±.04	2.16±.04
9	6.6	6.7	6.9	6.8	1.70±.05	1.99±.13	1.88±.04
10	7.0	7.1	7.1	7.1	1.83±.06	2.06±.12	1.91±.03
11	8.0	8.0	8.0	8.0	1.82±.03	2.09±.08	1.94±.04
12	9.0	9.0	9.0	9.0	2.23±.02	2.66±.05	1.47±.006
Dist. H_2O							

done in figure 2, we secure a curve having a double maximum with a minimum between at a pH of 5.75–5.8. The curves given in figure 2 are those for 12 and 48 hours.

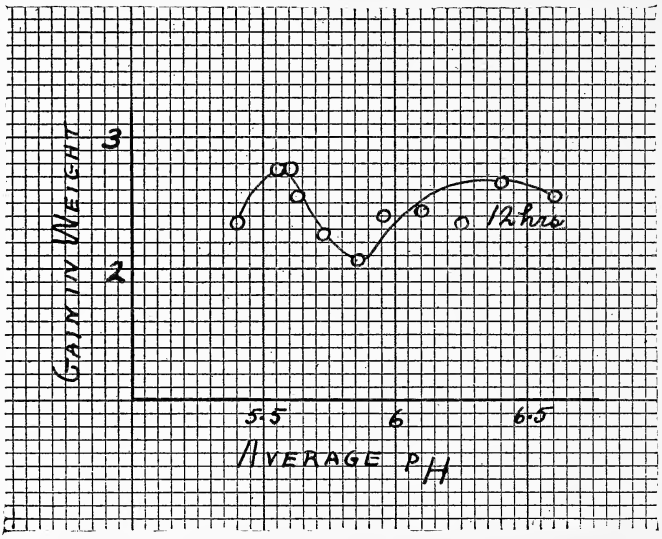

FIG. 3. The gain in weight of 10 g. of potato-tuber tissue in 150-cc. quantities of 0.01 M phosphoric acid and 0.01 M sodium hydroxide in 12 hours.

In the third experiment the dilute buffer mixtures were again used, but what appeared to be the critical region of hydrogen-ion concentration was covered with a greater number of solutions. In this case the reaction at the alkaline end of the series changed toward greater acidity. This change was evident in solutions of pH greater than 5.75. An examination of the in-

TABLE 3. *Gain or loss in weight of 10 g. of potato-tuber tissue in 6, 12, and 24 hours in 150-cc. quantities of buffer mixtures of 0.01 M Na citrate and 0.01 M NaOH, the buffer mixtures being replaced with fresh quantities at each weighing*

Sol. No.	Initial pH	pH after 6 Hrs.	pH after 12 Hrs.	pH after 24 Hrs.	Gain or Loss, 6 Hrs.	Gain or Loss, 12 Hrs.	Gain or Loss, 24 Hrs.
					g.	g.	g.
1	5.1	5.4−	5.4	5.3	0.158	−1.192	−1.925
2	5.2−	5.4	5.4	5.4	0.248	−1.410	−2.068
3	5.5	5.5	5.5	5.5	0.066	−1.608	−2.067
4	5.67	5.67	5.67	5.67	0.125	−1.525	−2.083
5	5.8	5.8	5.8	5.8	0.316	−1.258	−1.947
6	6.0	6.0	6.0	5.8	0.333	−1.242	−2.092
7	6.1	6.2	6.0	5.8	0.558	−1.017	−1.767
8	6.2	6.3	6.0	5.8	0.566	−0.825	−1.758
9	6.4	6.3	6.2	6.0	0.716	−0.750	−1.617
10	6.7	6.8	6.6	5.8	1.000	−0.125	−1.250
11 Redist. H_2O	6.8	6.6	6.7	6.4	1.566	1.442	1.583

creases in weight showed that they were comparable in amount to those secured in the former experiment with the more dilute buffer mixtures of H_3PO_4 and NaOH. In the first four solutions, pH 5.4–5.6, the potato continued to gain in weight for the 48 hours. Solutions 5–10, pH 5.75–6.6, showed losses in weight between the 24- and 48-hour weighings. Again it was noted that in the solution of original pH 5.4 the change in weight was

TABLE 4. *Gain or loss in weight of 10 g. of potato-tuber tissue in 6, 12, and 24 hours in 150-cc. quantities of buffer mixtures of 0.01 M KH phthalate and 0.01 M NaOH*

Sol. No.	Initial pH	pH after 6 Hrs.	pH after 12 Hrs.	pH after 24 Hrs.	Gain or Loss, 6 Hrs.	Gain or Loss, 12 Hrs.	Gain or Loss, 24 Hrs.	Ash, 24 Hrs.
					g.	g.	g.	g.
1	4.2	4.6	4.6	4.6	1.092	−0.900	−2.183	0.0111
2	4.6	4.8	4.7	4.7	1.242	−0.150	−1.933	0.0149
3	4.9	5.2	5.2	5.0	1.367	0.575	−1.408	0.0217
4	5.5	5.6	5.5	5.6	1.400	0.570	−0.983	0.0241
5	5.85	5.85	5.8	6.0	1.392	0.942	−0.008	0.0349
6	6.2	6.2	6.2	6.2	1.333	0.800	−0.492	0.0394
7	6.2+	6.2+	6.3	6.2	1.442	1.025	−0.267	0.0358
8	6.3	6.3	6.4	6.2	1.492	1.142	−0.050	0.0314
9	6.9	6.4	6.4	6.2	1.433	1.033	−0.108	0.0359
10	7.6	6.5	6.6	6.2	1.408	0.925	−0.150	0.0383
11 Redist. H_2O	6.6	6.2	6.3	5.8	2.097	2.008	1.550	0.0409

least between the 24- and 48-hour weighings. In figure 3 the gains in weight at the end of 12 hours are plotted against the average pH of the solutions. If a smooth curve is drawn through these points, we have again a double maximum with a minimum between at a pH of 5.85. If all points were connected, a W-shaped curve with the peak at 6.05 would be obtained.

Citrate—NaOH Series

The buffer mixtures used in this series were prepared by mixing 0.1 M secondary sodium citrate and 0.1 M NaOH as proposed by Sorensen following the tables given by Clark (3), and diluting the solutions so secured either 10 or 50 times. Redistilled water was used in preparing these buffer mixtures. The calculated concentrations of the secondary sodium citrate and sodium hydroxide used in these two dilute buffer mixtures are given in table 1.

In the first experiment the original buffer mixtures diluted 10 times were used. This was equivalent to mixing 0.01 M secondary sodium citrate and 0.01 M NaOH. The potato was weighed at the end of 6, 12, and 24 hours. At the first weighing the solutions from 4 to 9 were slightly turbid due to the development of bacteria, while the rest were clear. The potato appeared to be in good condition. The bacterial development was about the same at the second weighing, but the potato was quite flaccid especially at the

acid end. At the 24-hour weighing the development of bacteria was marked and the potato was very flaccid in all cases except in the redistilled water. The results of this experiment are given in table 3. In this case the presence of the potato shifted the pH toward greater alkalinity in the first two solutions, pH 5.1–5.2, and toward greater acidity in the last six, pH 6.0–6.8. In solutions 3, 4, and 5, pH 5.5–5.8, no change was noted.

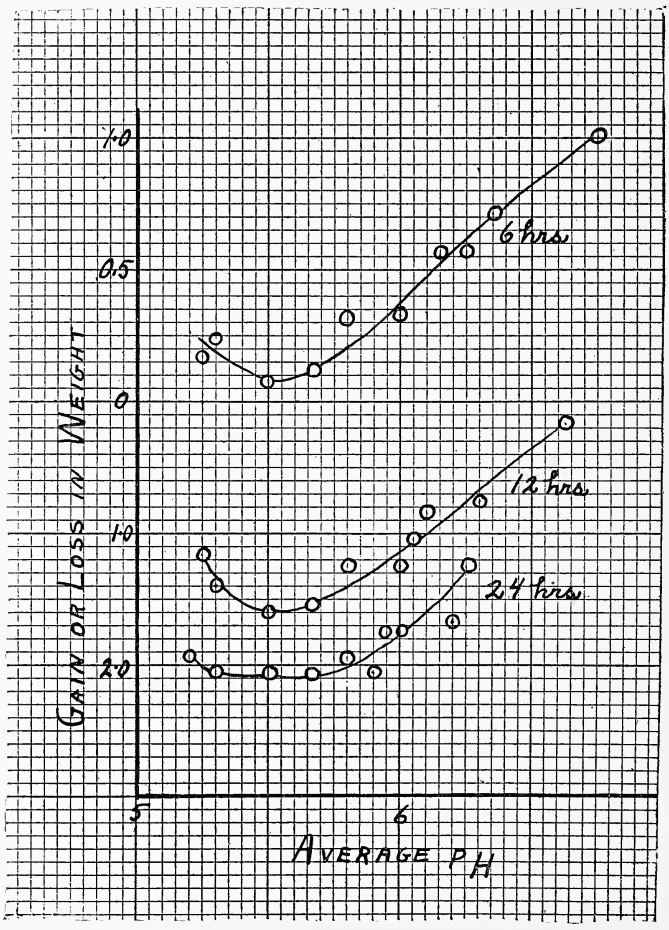

FIG. 4. Gain or loss in weight of 10 g. of potato-tuber tissue in 6, 12, and 24 hours in 150-cc. quantities of buffer mixtures of 0.01 M secondary sodium citrate and 0.01 M sodium hydroxide. Buffer mixtures replaced at 6, 12, and 24 hours. See table 3.

When the changes in weight were examined it was found that the increase in weight was considerably less than with the phosphate series of buffer mixtures. The potato in all the buffer mixtures showed losses by the end of 12 hours which increased in amount by the end of 24 hours. This was evidently due to the citrate ion, as the tendency for the increase in weight was to become greater or the loss in weight less as we proceed toward

the alkaline end of the series where the concentration of the citrate present was less. The loss between the 12- and 24-hour periods was least in solution 3, pH 5.5, where it was 0.46 g. as compared to losses of 0.658–1.125 g. in the other buffer mixtures. If we plot the gains or losses in weight against the average pH of the solutions, as in figure 4, we obtain a curve with a minimum at pH 5.5–5.67. This series would also probably have given us a double-maximum curve had we been able to continue it to greater extremes of acidity and alkalinity.

TABLE 5. *The amount of change in weight expressed as percentage of the total weight and as percentage of gain in weight found between the minimum and the acid or alkaline maximum in the experiments on water-absorption by potato-tuber tissue*

	pH of Acid or Alkaline Maximum	pH of Minimum	Time	Amt. of Change in Weight in Reaching Minimum	
				% Maximum Total Weight	% Maximum Gain
0.1 M H_3PO_4	4.85	5.75	8 hrs.	2.1	17
0.1 M NaOH	7.95	5.75	8 hrs.	3.8	28
0.01 M H_3PO_4	5.5	5.75	12 hrs.	4.9	26
	3.5	5.75	12 hrs.	5.5	29
0.01 M NaOH	6.2	5.75	12 hrs.	5.2	27
0.01 M H_3PO_4	5.6	5.85	12 hrs.	5.7	26
0.01 M NaOH	6.4	5.85	12 hrs.	5.1	24
0.01 M secondary Na citrate	5.3	5.5	6 hrs.	0.9	73
0.01 M NaOH	6.75	5.5	6 hrs.	8.5	93
0.002 M secondary Na citrate	5.6	5.8	6 hrs.	1.6	11
0.002 M NaOH	6.35	5.8	6 hrs.	2.1	10
0.01 M KH phthalate	5.55	6.2	6 hrs.	0.6	4
0.01 M NaOH	6.3	6.2	6 hrs.	1.4	11

A still more dilute series of citrate buffer mixtures was used because of the rapid incidence of loss in weight which took place in the stronger series used first. The second series comprised the original mixtures diluted 50 times instead of 10 times. This was equivalent to mixing 0.002 M secondary sodium citrate and 0.002 M NaOH. The potato tissue was weighed at the end of 6, 12, and 24 hours. The solutions at the end of 6 hours were clear, and the potato was in good condition. There was slight evidence of bacterial action at the second weighing, and, while most of the discs were in good condition, some flaccid ones were present in each lot. By the end of 24 hours all the solutions were turbid, indicating the growth of bacteria, and the potato was flaccid. In this case solutions 1, 2, 3, 4, and 5, pH 5.4–5.8, became more alkaline as a result of standing in contact with the potato, the change being greater in the more acid solutions. In solutions 6, 7, 8, 9, 10, pH 6.1–6.8, the shift was toward greater acidity, the greater change taking place in the solutions of higher alkalinity.

An examination of the changes in weight showed that much greater increases took place with this more dilute solution than were found with the stronger one. However, the maximum was past before the 12-hour weighing, and by 24 hours the potato in all the buffer mixtures save the most alkaline showed a loss in weight. This loss between the 12- and 24-

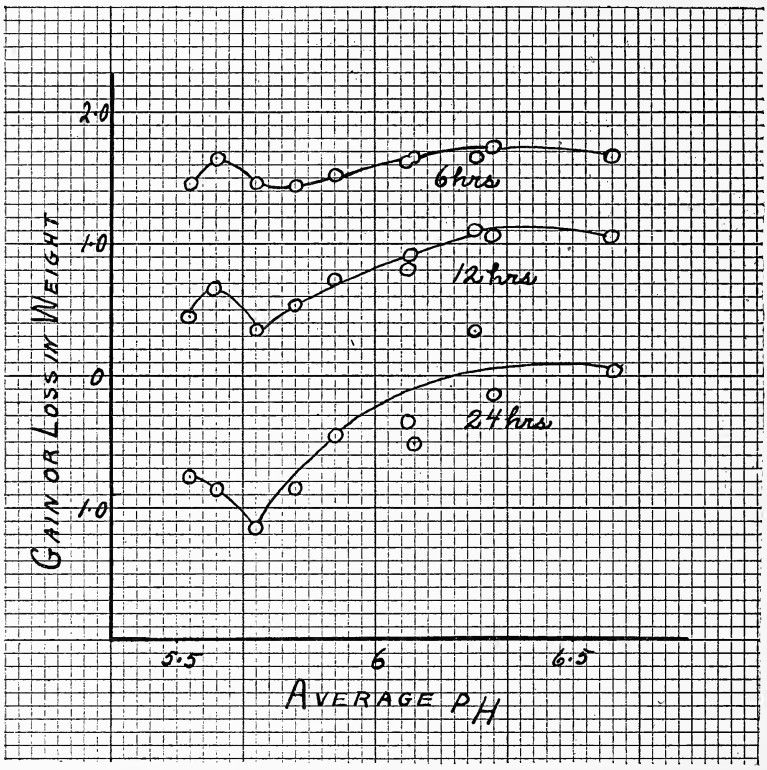

FIG. 5. Gain or loss in weight of 10 g. of potato-tuber tissue in 6, 12, and 24 hours in 150-cc. quantities of buffer mixtures of 0.002 M secondary sodium citrate and 0.002 M sodium hydroxide. Buffer mixtures changed at 6, 12 and 24 hours.

hour weighings was least in solutions 10, 6, and 5, pH 6.8, 6.1, and 5.8. Examining the curve of gain or loss in weight plotted against the pH (fig. 5), a minimum was found at 5.7–5.8, at the 6-, 12-, and 24-hour weighings. The curves at all three periods are much alike.

Phthalate—NaOH Series

The solutions obtained by mixing 0.1 M KH phthalate and 0.1 M NaOH as proposed by Sorensen, using the tables given by Clark (3), were diluted 10 times. The dilution raised the pH of the more acid solutions and lowered that of the more alkaline. The potato was weighed at the end of 6, 12, and 24 hours, and the data are presented in table 4. At the end of 6 hours,

when the first change in weight was measured, the solutions were clear and the potato was in good condition. At the time of the second weighing the solutions were clear, but the potato was flaccid in the acid solutions but turgid and in good condition in the alkaline solutions. At the end of 24 hours, solutions 3–11 were quite cloudy, indicating the growth of bacteria, and all the potato was flaccid save that in redistilled water. It can be noted in table 4 that solutions of pH 4.2–5.85 became more alkaline in contact with the potato, the greater change being at the acid end. Solutions of pH 6.2 and 6.3 showed no change, while the two most alkaline, pH 6.9 and 7.6, showed a decided change toward greater acidity. The changes in weight were quantitatively much the same as those found with the more dilute citrate buffer mixtures. By the end of 24 hours, the potato in all the buffer mixtures weighed less than it did originally. When the change in weight is plotted against the pH of the solution, a curve (figure 6)

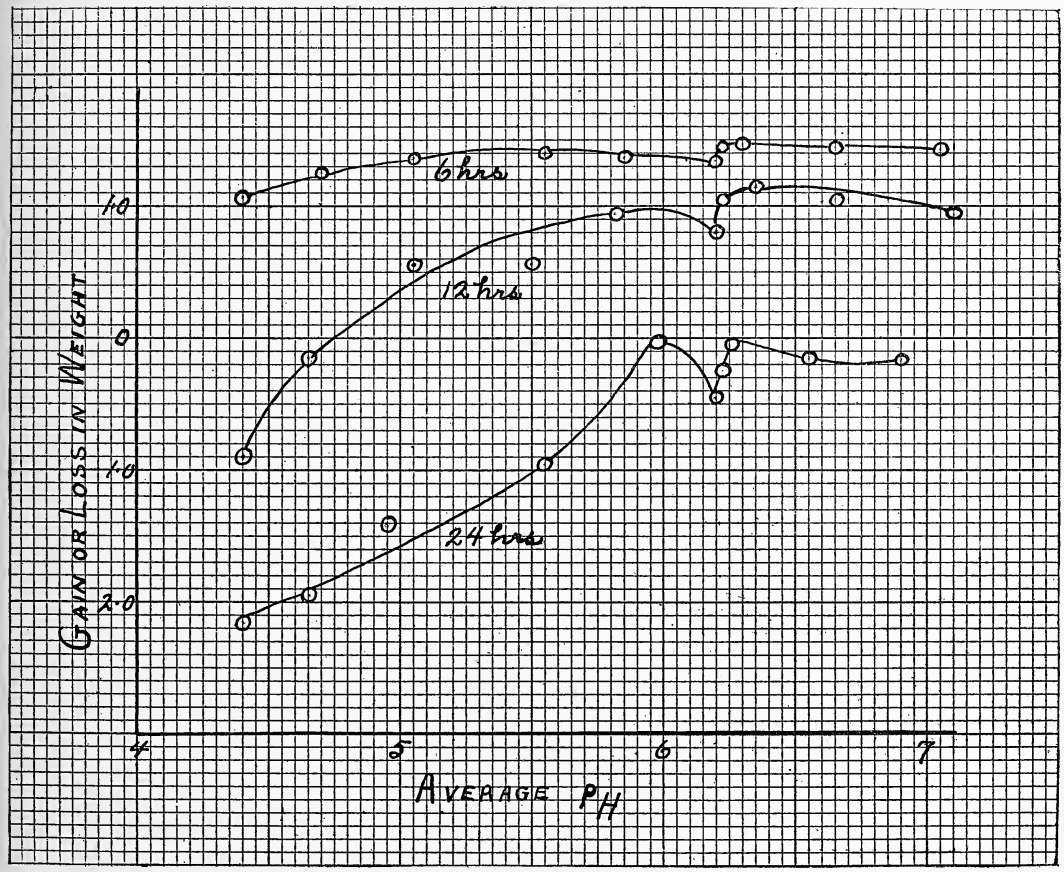

FIG. 6. Gain or loss in weight of 10 g. of potato-tuber tissue in 6, 12, and 24 hours in 150-cc. quantities of buffer mixtures of 0.01 M potassium hydrogen phthalate and 0.01 M sodium hydroxide. See table 4.

TABLE 6. *The regions in each experiment on water-absorption where no change in reaction took place, or where a change toward greater alkalinity or acidity occurred in the buffer mixtures, in consequence of the presence of the potato tissue*

Buffer Mixtures	Reaction Changed toward Greater Alkalinity in Solutions of pH	No Change in Reaction in Solutions of pH	Reaction Changed toward Greater Acidity in Solutions of pH
0.1 M H_3PO_4–0.1 M NaOH.....	3.5–5.4	5.8–7.9	
0.01 M H_3PO_4–0.01 M NaOH.....	4.1–5.4	5.8–8.0	
0.01 M H_3PO_4–0.01 M NaOH.....	5.4	5.6	5.75–6.6
0.01 M Na citrate–0.01 M NaOH.....	5.1–5.2	5.5–5.8	6.0–6.7
0.002 M Na citrate–0.002 M NaOH...	5.4–5.8	6.1	6.3–6.8
0.01 M KH phthalate–0.01 M NaOH..	4.2–5.5	5.85–6.3	6.9–7.6

TABLE 7. *The original pH values of the buffer mixtures used in the experiments with dyes, together with the pH of the solutions after standing in contact with the potato and the average; the pH was estimated where starred*

	Buffer Mixtures of 0.02 M H_3PO_4–0.02 M NaOH			Buffer Mixtures of 0.002 M H_3PO_4–0.002 M NaOH.					Buffer Mixtures of 0.002 M Na Citrate–0.002 M NaOH		
Sol. No.	Original pH	Final pH	Ave. pH	Original pH	Final pH, 3 Hrs.	Ave. pH for 3 Hrs.	Final pH, 5 Hrs.	Ave. pH, 5 Hrs.	Original pH	Final pH, 6 Hrs.	Ave. pH
Redist. H_2O											
1	6.5	6.3	6.4	6.6	6.4	6.5	6.4	6.5	6.4	6.3	6.35
2	2.0*	2.0*	2.0*	2.0*	3.7	2.85	4.1	3.05	5.4−	5.4	5.4
3	3.1	3.5	3.3	4.2	5.6	4.9	5.7	4.95	5.4	5.5	5.45
4	4.6	5.1	4.85	5.6	5.8	5.7	5.9	5.75	5.67	5.67	5.67
5	5.6−	5.6	5.6	5.8	5.9	5.85	5.8	5.8	5.8	5.8	5.8
6	5.8+	5.8+	5.8+	6.1	6.1	6.1	5.8	5.9	6.2	6.2	6.2
7	7.0	6.9	6.95	7.0	6.8	6.95	6.4	6.7	6.3	6.2	6.25
8	8.0	7.6−	7.8	7.7	7.6	7.65	6.8	7.25	6.5−	6.5	6.5−
9	9.0 above	8.05	8.52 above	7.9−	7.6	7.75	6.9	7.4	6.6	6.4	6.6
10	9.75+	8.15	8.95	8.1	7.6	7.85	6.8	7.45	6.7	6.6	6.65

is obtained with a minimum at pH 6.2–6.25. The curves for the three intervals of time are much alike in shape.

A consideration of the six experiments described permits the following statements by way of summary. When discs of potato-tuber tissue approximately 1 x 15 mm. were soaked in buffer mixtures composed of H_3PO_4 and NaOH, secondary sodium citrate and NaOH, or potassium hydrogen phthalate and NaOH, the change in weight plotted against the H-ion concentration of the solutions expressed as pH produced a curve having a double maximum with a minimum between. With the H_3PO_4-NaOH series this minimum was located at a pH of 5.8–6.0; with the secondary

sodium citrate-NaOH series the minimum was found at pH 5.5–5.7; and with the potassium hydrogen phthalate-NaOH series the minimum was located at a pH of 6.2–6.25. In all three sets of the H_3PO_4-NaOH series, the least change in weight occurred in the solutions having a pH of 5.4. This was also true in one experiment with the secondary sodium citrate-NaOH buffer mixtures. The shapes of the curves were in each case much the same for all three weighings at 8, 12, and 24 hours, 12, 24, and 48 hours, or 6, 12, and 24 hours. The shape of the curve and the location of the minimum point for the turgid tissue at the end of 6 hours was the same as for the flaccid and presumably dead tissue at the end of 24 hours in the citrate and phthalate series.

The differences in weight between the potato in the solutions where the minimum weight was found and that of the potato in the solutions producing the maximum weight on the acid or alkaline sides of the minimum were not great. As shown in table 5, they ranged from 0.6 percent to 8.5 percent of the maximum total weight. This difference appears insignificant. Since, however, a large percentage of water must be present in resting tissue, it would appear that the difference expressed as percentage of gain rather than percentage of total weight might be of greater physiological importance. It is the small additional amount of water absorbed that is responsible for increased turgidity and for the phenomena which accompany it. If we consider the differences in weights from this standpoint they become more significant. Expressed as percentage of maximum gain, the majority of the differences were in the neighborhood of 25 percent. In other words, the gain of the potato tissue in solutions showing the minimum point was about 25 percent less than it was where the maximum gain was found on either side.

When the amount of the differences in gain in weight for the potato at the minimum and at the maximum on either side for the different buffer mixtures is considered, it would appear that in the phosphate series the more dilute the buffer mixtures of a given set the greater the difference. Thus, at the end of 12 hours the differences for the 0.1 M series and the two 0.01 M series between the acid maximum and the minimum were 0.29 g., as compared to 0.59 or 0.67 g. and 0.73 g. respectively. For the alkaline maximum and the minimum the difference was 0.32 g. compared to 0.64 g. and 0.41 g. With the citrate series the situation is complicated by the greater toxicity of the citrate ion.

Attention should also be called to the fact that it was only with the 0.002 M H_3PO_4-0.002 NaOH sets of buffer mixtures that gains as great as those found in distilled or redistilled water were observed.

A consideration of the change in reaction which the buffer mixtures showed as a result of contact with the potato indicates in general that up to pH 5.5–5.8 the solutions became more alkaline, while in solutions more alkaline than pH 5.5 or 6.1 they remained unchanged or became more acid. The results of the changes which occurred in the reaction of the buffer mixtures due to the presence of the potato are summarized in table 6.

Discussion

A consideration of the results of the foregoing experiments shows that the analogy between the absorption of water by such a protein as gelatin in solutions of different hydrogen-ion concentrations and the absorption of water by potato-tuber tissue under the same conditions is good if we assume that the isoelectric point of the potato tissue is in the vicinity of pH 6.0. In each case the water absorption by the potato tissue is at a minimum in the vicinity of pH 6.0 and increases in amount as we proceed toward greater alkalinity or greater acidity, passing through a maximum and then decreasing. This is what would be expected if the potato tissue acted as gelatin does and had an isoelectric point in the vicinity of pH 6.0. The shifts in the reaction of the buffer mixtures resulting from contact with the potato, to which attention was called in describing the individual experiments, are also what would be expected. As was pointed out in summarizing the experiments, those buffer mixtures in the vicinity of pH 6.0 (5.5–6.2) showed little or no change in reaction due to the presence of the potato. Those more acid showed changes in general toward greater alkalinity, and when those more alkaline than about pH 6.0 showed a change it was toward greater acidity. On the acid side of the isoelectric point a protein reacts with anions, with the result that the reaction of the solution in which the protein is placed should shift toward greater alkalinity. On the alkaline side of the isoelectric point it reacts with cations, resulting in a change of the reaction of the solution toward greater acidity. Part of the change in reaction in the alkaline solutions was probably due to the production of carbon dioxide by the potato tissue.

Two apparent difficulties prevent the analogy from being complete. The first of these is the fact that the position of the minimum point of water absorption, which should be the isoelectric point, is apparently affected by the acid radicle of the buffer mixture. Thus, with the phosphates it was apparently located at pH 5.8, with the citrate series at 5.5–5.7, and with the phthalate series at 6.2. These differences are apparently too great to be accounted for by errors in the determination of the hydrogen-ion concentration. It should be pointed out, however, that Michaelis and Rona (17) found that anions shifted the pH most favorable for precipitation of denatured albumin toward the acid side and that cations shifted it toward the alkaline side.

A second unexpected but interesting fact is that the dead tissue showed the same minimum for water absorption as the living tissue. This might suggest that it was not the living protoplasm with which we were dealing, but that either dead protoplasm killed in cutting the potato or non-living protein contained in the cells was concerned in both the living and the completely dead material. There is no complete evidence to demonstrate the truth of either of these possibilities. It would appear, however, that the differences obtained in the experiments with the dilute H_3PO_4-NaOH

buffer mixtures were too great to be accounted for by assuming that dead protoplasm or non-living protein was involved. Of course a comparatively small change in the isoelectric point with death would not be revealed by the methods used. Michaelis and Davidsohn (16) measured the isoelectric point of genuine albumin and of albumin denatured by heat by precipitation with sodium acetate. They found that the denaturing shifted the isoelectric point from pH 5.39 to pH 4.69.

MacDougal (14) has reported, in contradiction to earlier statements by the same author, that purified agar swells more in dilute HCl, pH 4.2, and dilute KOH, pH 11, than in water. The agar at 0.75 percent which is near the limit of its gelation at 15° C. had a pH of 6.5. These results indicate that pentosans may also show a double-maximum curve when their swelling is plotted against their pH. The confirmation of this possibility would permit an analogy to be drawn between the water absorption by potato and that by a pentosan. This analogy, with our present knowledge, would not hold for the changes in reaction which the potato produced in the buffer mixtures, nor would it explain the results obtained on the absorption of dyes reported later in this paper. Rosa (21), in some incomplete studies made in this laboratory, did not confirm MacDougal's results.

The Absorption of Dyes by Plant Tissue

It was pointed out in discussing the relation of the isoelectric point to the properties of gelatin that the amphoteric gelatin on the acid side of the isoelectric point forms salts with anions only, existing as gelatin chloride, gelatin sulfate, gelatin citrate, etc., while on the alkaline side of the isoelectric point it forms salts with bases existing as sodium gelatinate, potassium gelatinate, calcium gelatinate, etc. Since the colored radicles of acid dyes like eosin are in the acid radicle, gelatin of different hydrogen-ion concentrations takes up and retains the dye on the acid side of the isoelectric point and does not take it up or does not retain it on the alkaline side of the isoelectric point. With basic dyes like safranin, on the other hand, gelatin will be stained on the alkaline side of its isoelectric point (pH 4.7) and will not be stained on the acid side of pH 4.7. This method of determining the isoelectric point has been used by Loeb (13) for gelatin and by Thomas and Kelly (24) for the protein, collagen, of hide powder.

In applying the dye method to the investigation of the isoelectric point of plant tissue, potato-tuber tissue was used chiefly and found most satisfactory. A few experiments were also carried out with Spirogyra, leaves of Elodea, and sections of tomato and Kudzu stems. In general it was found that plant tissue which had been treated with buffer mixtures of different H-ion concentration responded to dyes as though it had an isoelectric point.

The potato used in these experiments was prepared as previously described for the experiments on water absorption. From 2 to 25 discs of

potato, 1 x 15 mm., were placed in tumblers in 200-cc. quantities of the buffer mixtures of different H-ion concentration. After periods varying from 1.5 to 5 hours, two discs from each solution were removed to a Syracuse watch crystal. They were covered with the dye, and after a few seconds' to 15 minutes' staining were removed, washed with redistilled water, and examined for relative intensity of staining. In some cases, after staining the pieces were washed with the original buffer mixtures. Water redistilled from alkaline and acid permanganate was used throughout in the preparation of the buffer mixtures and the solutions of the dyes.

Three sets of buffer mixtures were used, two composed of H_3PO_4 and NaOH and one of secondary sodium citrate and NaOH. Most of the experiments were performed using solutions obtained by mixing 0.1 M H_3PO_4 and 0.1 M NaOH and diluting 50 times. This was equivalent to mixing 0.002 M H_3PO_4 and 0.002 M NaOH. The original pH of these solutions as well as the pH of the solutions after 3 and 5 hours' contact with the potato are given in table 7. A set of buffer mixtures was also prepared by mixing 0.1 M H_3PO_4 and 0.1 M NaOH and diluting 5 times. This was equivalent to mixing 0.02 M H_3PO_4 and 0.02 M NaOH. The initial pH of the solutions used in this set and the pH of the solutions after standing in contact with the potato are also given in table 7. Buffer mixtures obtained by mixing 0.1 M secondary sodium citrate and 0.1 M NaOH and diluting 50 times were also used. This was equivalent to mixing 0.002 M secondary sodium citrate and 0.002 M sodium hydroxide. The original pH values of these buffer mixtures and the pH of the solutions after contact with the potato are given in table 7.

A consideration of the data there given confirms what was previously observed in the experiments on water absorption. The presence of the potato changed the pH of the solutions on the acid side of pH 5.8 toward greater alkalinity and on the alkaline side of 6.1 or 6.2 toward greater acidity.

The acid dyes used were orange G, eosin, Martius yellow, and acid fuchsin. The basic dyes were methylene blue, safranin, malachite green, and crystal violet.

Excellent results were secured with the acid dyes, eosin and Martius yellow, the potato responding as though its isoelectric point were located somewhat above pH 6.0.

The eosin was used at a concentration of 1–500 and the periods of staining varied from 3 to 10 minutes. To cite specific experiments:

Twenty slices of potato were soaked in the buffer solutions composed of 0.002 M H_3PO_4 and 0.002 M NaOH for 2 hours. Two slices from each buffer mixture were removed and stained in Syracuse watch crystals with 1–500 eosin for 3 minutes. When removed from the stain little difference in color was noted. They were washed in the watch crystals with 3 changes of redistilled water. The potato from solution 2, pH 2.85, was very red;

that from solutions 3, 4, 5, and 6, pH 4.9–6.1, less red than 2 but distinctly redder than that from 7, 8, 9, and 10, pH 6.95–7.85. The potato from the redistilled water, pH 6.5, was about like that from solution 6. The potato discs from solutions 3, 4, 5, and 6 were almost alike, with some tendency to greater intensity of staining in those from the more acid solutions. The potato discs from solutions 7, 8, 9, and 10 were almost alike. With 10 minutes' staining but more thorough washing, even better results were secured.

These results show that the acid dye eosin is absorbed and held by potato-tuber tissue from buffer mixtures whose average pH is 6.1 or less, and is held very weakly by potato discs from buffer mixtures of average pH 6.95 or above. In other words, the potato acted much as we should expect a protein to act with isoelectric point between pH 6.1 and pH 6.95.

Similar results were secured with the buffer mixtures made from 0.02 M H_3PO_4 and 0.02 M NaOH. After 3 hours' contact with the buffer mixtures, the potato was stained with eosin 1–500 for 5 minutes. The potato from solutions 2, 3, 4, 5, and 6, pH 2.0–5.6, was distinctly deeper red than that from solutions 7, 8, 9, and 10, pH 6.95–8.95 +. The line of separation appeared between 6 and 7.

With the citrate set of buffer mixtures, the potato was stained with 1–500 eosin for 10 minutes after 6 hours' treatment with the buffer mixtures. The intensity of color decreased from that of the potato from solution 2 to that of the potato from solution 10. The potato from the redistilled water stained about like that from solution 5. There was a fairly sharp line of separation between the potato from solution 5, pH 5.8, and that from solution 6, pH 6.2. However, the potato discs from solutions 6 and 7 were a little redder than those from 8, 9 and 10, which latter were about alike. This would place the isoelectric point either between pH 6.2 and pH 6.3 or between pH 6.3 and pH 6.5.

A number of experiments were also completed with Martius yellow, using the 0.002 M H_3PO_4–0.002 M NaOH buffer mixtures only. For example, the potato was treated with buffer mixtures composed of 0.002 M H_3PO_4 and 0.002 M NaOH for 2 hours. The pieces were removed and stained with the Martius yellow 1–2500 for 5 minutes. They showed little difference in intensity of staining at this time. They were washed with redistilled water several times and with the buffer mixtures for 20 minutes. An examination of the discs showed that the potato responded as would be expected if an isoelectric point existed between pH 6.1 and pH 6.95. The potato from the redistilled water stained somewhat less than any of the other pieces.

Orange G at a concentration of 1–500 for 1 minute and for 10 minutes was also used. Most of the stain, however, washed out, leaving only a little color in the pieces from the more acid solutions. A longer period of staining or a stronger solution of the dye might give more favorable results.

Acid fuchsin was another dye which did not yield as good results as eosin or Martius yellow. Used at a concentration of 1–4000 for 10 minutes, the staining was too weak to permit conclusions to be drawn. Used at a concentration of 1–500 for $2\frac{1}{2}$ minutes, on potato which had stood in the buffer mixtures of 0.002 M H_3PO_4 and 0.002 M NaOH about 6 hours, better results were secured. The pieces of potato did not stain uniformly with this dye, but irregularly, the vascular bundles taking the stain deeply. The potato discs from the acid solutions stained more heavily than those from the alkaline side, the line of separation appearing between solutions 6 and 7 as for eosin and Martius yellow. The potato from the redistilled water stained about like that from solution 6. The potato from solution 2 showed the heaviest staining.

The experiments with the basic dyes were considerably less satisfactory than those with the acid dyes. The potato from the different buffer mixtures, as well as that from the redistilled water, took the basic stains so uniformly that little difference in intensity of staining could be noted in the potato from the different buffer mixtures. This was at first very puzzling. Microscopic examination of the potato stained with safranin or crystal violet, however, showed that these dyes stained the starch grains and cell walls heavily. The hydrogen-ion concentration of the solutions in which the potato had stood apparently did not noticeably affect the intensity with which the starch combined with the dye. The intensity with which the starch and cell walls took up the dye covered up to a large extent any other effect present. With eosin the starch grains were not stained, and only the protoplasmic content of the cell took the dye to any considerable extent. This offers what appears to be a satisfactory explanation for the better results secured with the acid dyes. However, sufficient positive results were secured with safranin, crystal violet, and methylene blue to satisfy us that the basic dyes were taken up more strongly by potato from the solutions of pH 6.95 and above.

Confirmation of the conclusion that the basic dyes were taken up and held more strongly by the potato from the alkaline solutions was obtained by the way in which the stain was lost from the potato after staining. Thus, discs of potato, after standing 2 hours in the 0.002 M H_3PO_4–0.002 M NaOH buffer mixtures, were stained for 5 minutes with methylene blue 1–18000. The pieces were a uniform blue after staining. They were then laid in 15 cc. of the original buffer mixtures. After 2 hours the pieces from solutions 4, 5, and 6 were somewhat lighter in color than those from 7, 8 and 9. The difference, however, was not great. The buffer mixtures in which the stained potato had been washed were poured into test tubes, and the intensity of color was compared in the comparator. Those from the acid end were a deeper blue than those from the alkaline end. Thus 3, 4, and 5 combined were a much deeper blue than 7, 8, and 9; 4 and 5 were much bluer than 7 and 8. Little difference could be noted between 7 and 8 as

compared with 9 and 10. The stained potato was then replaced in 10-cc. quantities of the buffer mixtures and allowed to stand for 16 hours. When removed from the solution all pieces were colorless, in consequence of a reduction of the methylene blue. The exposure to the air and treatment with H_2O_2 developed the blue color. The pieces from the alkaline buffer mixtures were bluer than those from the acid buffer mixtures, although a sharp line of separation such as could be made in the case of the eosin-stained potato could not be made. The potato from solutions 2, 3, 4, and 5 was lighter blue than that from solutions 7, 8, 9, and 10, but the pieces from solution 6 were intermediate between those from 5 and 7.

Clearer-cut results with the basic dyes might be secured by weaker staining and with more thorough washing with the buffer mixtures after staining. The use of tissue with little or no starch and with large thin-walled cells well filled with protoplasm would also be advisable.

The response of dead potato was also compared with that of living potato, using the 0.002 M H_3PO_4–NaOH buffer mixtures. It was expected that a distinct difference would be found between the response of the dead and that of the living potato. The potato was killed by treatment with 50 percent alcohol. The living and the dead potato were treated with the same buffer mixtures for the same length of time and stained in the same dishes. No difference qualitatively was noticed between the dead and the living material. The dead potato, however, stained more deeply than the living potato.

Experiments were also carried out with Spirogyra with methylene blue and orange G. Difficulties at once arose, due to lack of uniformity of the material and the trouble of comparing the intensities of color in microscopic preparations. The Spirogyra was placed in buffer mixtures of 0.002 M H_3PO_4–0.002 M NaOH to which methylene blue at a concentration of 1–100,000 was added at once or after a period. The results of 5 experiments indicated that the greatest intensity of color was found in the Spirogyra from solutions 8, 9, and 10, that is, from solutions having a pH of 8.0 or more. Variations in the staining of duplicate lots and the difficulty of comparing the color intensity made accurate conclusions impossible. The same was true in experiments with orange G, although no difficulty was found in determining that the orange G was taken up more strongly from the acid solutions. Sprigs of Elodea, instead of Spirogyra, or sections of young tomato stems or Kudzu plants were also found unsatisfactory material. The small size of the sections and the presence of the natural green pigment made the difficulties too great to encourage experimentation with them.

To summarize briefly the results secured with the experiments on dyes: Discs of potato 1 x 15 mm. which were allowed to stand in 0.002 M H_3PO_4–0.002 M NaOH buffer mixtures of varying hydrogen-ion concentration for from 2 to 6 hours took up and retained acid dyes like eosin more strongly when the buffer mixtures had a pH of 6.1 or less than they did when the pH

of the buffer mixtures was 6.95 or more. Dead potato responded to a greater degree to staining, but qualitatively showed the same results. Using buffer mixtures of 0.002 M secondary sodium citrate and 0.002 M NaOH, the greater amount of dye was retained by those pieces from solutions of pH 6.2–6.3 or less while it was lost in washing by those pieces from solutions of pH 6.3–6.5 or greater. The acid dyes did not combine to any extent with the starch grains found in the cells of the potato-tuber tissue. With basic dyes the starch grains were stained, and pieces of potato from buffer mixtures of 0.002 M H_3PO_4 and 0.002 M NaOH stained rather uniformly. However, with care in staining and washing, it was demonstrated that basic dyes like safranin, crystal violet, and methylene blue were retained more strongly by potato from the solutions of pH 6.95 or greater than by those from solutions of pH 4.9–6.1.

Discussion

The analogy between the staining qualities of potato treated with different buffer mixtures and that of a protein like gelatin is good if we assume the isoelectric point of the potato-tuber tissue to be near pH 6.0. The potato-tuber cells, containing other materials like carbohydrates such as starch and pentosans and fat globules, and being surrounded by a cellulose wall, could not be expected to give such sharp and clear-cut results as the ash-free gelatin used by Loeb. The action of the basic dyes illustrates this point. It should be noted here that the position of what appears to be an isoelectric point as found by the method of water absorption agrees very well with that found by the dye method. In both cases it is located in the vicinity of pH 6.0. Attention should also be called to the fact that the death of the tissue, so far as these experiments gave evidence, did not affect the position of the isoelectric point.

General Discussion

The results which are reported in this paper on the absorption of water and on the absorption and retention of acid dyes by potato-tuber tissue can be explained by the assumption that an ampholyte, possibly a protein, plays the chief part in these processes and that its isoelectric point is in the vicinity of pH 6.0. The absorption of basic dyes by potato-tuber tissue is complicated by the fact that the starch, the cell walls, and possibly other constituents of the cell such as the pentosans, combine with the basic dyes at all the reactions involved. It is of course difficult to conceive of protoplasm as consisting of but one ampholyte with a single isoelectric point. The point to which we have called attention here may represent the resultant of the isoelectric points of several cell constituents.

While it is not considered that the hypothesis that potato-tuber tissue acts, in the processes indicated, like an ampholyte with an isoelectric point,

has been established by these experiments, it would appear very difficult to explain the phenomena which have been described on the basis of the conception of the cell as an osmotic chamber and of the theories of permeability of the plasma membrane which have been suggested. It would appear of extreme importance to reinvestigate the problems of water absorption and permeability, particularly with relation to salts and dyes, from this standpoint, bearing in mind that other constituents of the cell than proteins may react with dissolved material.[2]

Some substantiation of the conception that plant tissue may have an isoelectric point is afforded by the work of Osterhout on the conductivity of the tissue of Laminaria. Osterhout (19) found that alkali increased the conductivity of the tissue. Acid (20) was found to produce first a sharp decrease in conductivity, followed by a rapid increase. While no measurements were made on the hydrogen-ion concentrations of the solutions used, this result would be analogous to the changes which would take place in the conductivity of a protein which was originally in a solution more alkaline than the isoelectric point.[3] In the presence of alkali, the formation of greater and greater quantities of metal proteinates, accompanied by increased conductivity, would result. The addition of acid would first cause a decrease in the quantity of metal proteinates and probably produce an exosmosis of bases. When the isoelectric point was reached the minimum conductivity would be found. Further increase in acid would increase the amount of protein combined with anions and result in increased conductivity. It would appear advisable to reinvestigate the experiments which have been performed by Osterhout on the effect of salts and other conditions on the conductivity of plant tissue from the standpoint of the quantities of material which can conduct electricity rather than of the ease with which the electrolytes can move through the protoplasm.

The conception that protoplasm acts like an ampholyte with an isoelectric point in water absorption and in the absorption of solutes is one which, if substantiated by further investigation, would be of far-reaching importance. It would bear directly on the problem of water- and salt-absorption by plant cells, of the excretion of water by cells, the translocation of salts from one part of the plant to another, and conceivably on the rest period of plants in such cases as are apparently due to protoplasmic condition. (See Eckerson, 7.)

Whether we are correct or not in proposing the existence of an isoelectric point for plant tissue,[4] it would appear that the demonstration that there is a

[2] The analogy between the absorption of water by animal tissue and by proteins has been most completely presented by M. H. Fischer, in "Oedema and Nephritis," 3d ed.

[3] This has been suggested by W. O. Fenn. Similarity in the behavior of protoplasm and gelatine. Proc. Nat. Acad. Sci. 2: 537–543. 1916.

[4] From the effect of salts in acid and alkaline solutions on the absorption of dyes by Elodea, an isoelectric point of between p_H 3.8 and p_H 4.1 was proposed for the protoplasm of that plant by J. Endler. *See* Ueber den Durchtritt von Salzen durch das Protoplasma.

double-maximum curve with a minimum between for water absorption by plant tissue in solutions of different hydrogen-ion concentration offers a satisfactory explanation for the double-maximum curves found in the germination of spores and seeds, the growth of plants, and the infection of plants by fungi in media of different hydrogen-ion concentrations. The connection between water-absorption and spore germination or seed germination, and water-absorption and the growth of plants, is generally recognized. Recent evidence seems to indicate that the infection of plants by fungi which enter through the unpierced epidermis is due to a pressure process. A minimum water absorption would mean a minimum pressure and a minimum infection.

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STUDIES OF *LYTHRUM SALICARIA* I. THE EFFICIENCY OF SELF-POLLINATION

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The conditions that exist in species with trimorphic flowers permit the investigation of the problems regarding the nature of sex-differentiation and the degrees of compatibility between male and female organs under very favorable circumstances. In these forms the morphological adaptations for cross-pollination are often decidedly correlated with physiological incompatibilities which make cross-fertilization more certain by excluding the functioning of the chance self-pollinations which occur.

In general it is to be recognized that sexual fusions are favored by similarity of the gametes both in genetic constitution and in immediate origin, and that such conditions as trimorphism and self-incompatibility are to be regarded as, in a high degree, secondary and acquired. While these conditions secure the advantages of bringing together gametes of different origins and in greater or less degree of different genetic constitution, they decidedly limit and restrict free fertilization and full productivity.

The combination of morphological trimorphism with physiological incompatibilities as seen in species like *Lythrum Salicaria* may well be regarded as the highest degree of specialization in sex-determination and fertilization that exists in flowering plants. For this species there is the obvious morphological differentiation giving three lengths of pistils and three sets of stamens of lengths corresponding to those of the pistils, with the stamens bearing pollen differentiated as to size, color, and nature of the reserve food material in storage. For the individual, the flower of any particular plant has a pistil of one of the three lengths and a set of stamens for each of the other two lengths. This gives differentiation of forms as such, and in the single plant there is the differentiation that gives two kinds of stamens. Furthermore, this morphological differentiation is decidedly correlated with physiological differentiation. The noteworthy researches of Darwin (1865, 1877) showed that there is marked or even complete sterility to (1) self-pollinations, to (2) intra-form cross-pollinations, and to (3) the inter-form cross-pollinations that are illegitimate (*i.e.*, those that involve different lengths of pistil and stamen). Seed-production was hence found to be more or less limited to legitimate pollinations, which are necessarily crosses.

The specializations in these forms allow no doubts as to their significance such as have often been raised in regard to the colors of entomophilous flowers, for here the adaptations are morphological and depend directly

on the agency of insects for their effectiveness in pollination. The relative lengths of the different sets of stamens and of the pistils are unquestionably provisions favoring crossing.

It is, however, obvious that such differentiations are not fundamental expressions of sexual antithesis, since they are all reciprocal in any pair of flowers. Any two plants of any two forms are cross-compatible or cross-incompatible according to whether the cross is legitimate or illegitimate. The differentiations, at least in respect to maleness, that in dimorphic plants are seen in individuals as such and which appear to have definite genetic value are here seen equally well in the two sets of stamens in a single flower. All this emphasizes the fact that the conditions are secondary and acquired in contrast to the more primitive condition of homomorphism and a more general compatibility of gametes.

The generally accepted view has been that the differentiations in this species are well established and very stable. The tendency has been to emphasize, as did Darwin, the evidence that here there is adaptation favoring crossing, and to pass the evidence, which has to some extent been noted, that the adaptations are incomplete. It is to be recognized that such evidence has a very direct bearing on questions of the origin of trimorphism, of the nature of sex-differentiations, and of whether there is still opportunity for further selection in the species either toward greater or toward less restriction of fertilization. It is evidence along these lines that the writer wishes to present in reports, of which this is the first, of investigations with the species.

The Efficiency of Self-pollination for Plants Grown in Isolation

The writer's studies of *Lythrum Salicaria* were begun in 1917 in testing the self-compatibility of plants grown in isolation from other plants of the same or of related species. On such a plant hundreds of flowers open daily during a rather extended period of time and insect visitors can go from flower to flower, but with no chance, if the isolation is complete, of bringing pollen from other plants of *Lythrum Salicaria*. A large number of flowers are thus involved in the chance for self-fertilization (including here autogamy and geitonogamy), and the results can be obtained for the entire period of bloom. This test does not, however, determine the relative fertility of a plant to pollen of its two sets of stamens, nor does it reveal the need or the efficiency of particular species of insects in the self-pollination of the various forms, which may indeed give results that are highly variable from season to season or from year to year or according to location. However, if seed is produced there is positive evidence of self-compatibility, and the negative results may be tested further by controlled pollinations.

Short-styled Plants Grown in Isolation. Two large, well-developed plants several years old were dug from a mixed population growing at the New York Botanical Garden. One (*S no. 2*) was grown in the garden of the

Department of Botany at Columbia University, under the observation of Professor R. A. Harper, and one (*S no. 1*) at the University of Missouri under the care of Professor G. M. Reed. Both plants made vigorous growth and bloomed profusely, but neither plant produced a single capsule during the entire period of bloom of the season of 1917. In 1918 the plant at Missouri suffered severely from drought, and its failure to set any seed that year is not to be considered as adequate evidence of complete self-incompatibility.

The short-styled plant at Columbia University thrived and in the course of its season of bloom in 1918 produced 17 pods. Ten of these contained only mere rudiments of seeds, two contained one good seed (plump and apparently fully developed) each, three had two good seeds each, one had seven seeds, and one had eight. Sixteen of these 23 seeds germinated. In the following year (1919) this plant bloomed more abundantly than in the previous year and produced at least 5000 flowers, and during the entire season 161 pods matured. The seeds in 100 of these pods were counted; the number per pod ranged to 116, and the average was 23.67 (see table 1). This plant grew poorly in 1920, when its roots were separated to make two plants. In 1921 these thrived, and there was abundant bloom but only about 25 pods were produced. The irregular pod production by this plant may involve one or more of several conditions; possibly in 1919 insects may have brought pollen from a distance from plants of this species growing in city parks, or the irregularity may involve the presence or absence of certain insects that are most efficient in causing self-pollination.

Long-styled Plants Grown in Isolation. A long-styled plant (*L no. 1*) was grown at Baraboo, Wisconsin, under the care of Mr. William Toole, Sr., a well known pansy specialist. Plants of the variety *rosea* were also growing in the nursery at some distance away, so that some of the seed produced by the plant *L no. 1* may have been due to cross-pollination by insects. But another long-styled plant (*L no. 2*) was grown in what was certain isolation at Pleasantville, N. Y., under the care of Dr. M. A. Howe. Capsules were produced by both these plants. Of the 65 capsules on *L no. 1* in 1917, 16 contained no seeds, 17 contained one seed each, and the highest number of seeds in any capsule was 17. In 1917 a total of 53 capsules matured on the plant *L no. 2*. As shown in table 1, the number of seeds per capsule for this plant was also low, although all but three of the capsules contained some fully matured seeds.

In 1918 both plants produced capsules quite as in 1917, but the capsules on *L no. 2* were somewhat more numerous than in the previous year though still constituting a small proportion of the entire number of flowers.

The capsules produced by these two plants were distributed irregularly but rather indiscriminately throughout the flowering branches. Self-fertilization appeared to be effected with the same frequency and efficiency throughout the flowering period. No very decided seasonal differences appeared in respect to the proportional number of flowers which matured

TABLE I. *Self-compatibility in Lythrum Salicaria to insect-pollination, and cases typical for the results obtained for controlled hand-pollinations*

	Number of Flowers Pollinated	Number of Pods Produced	Number of Pods used in Seed Counts	Distribution of Seed per Pod. Class Groups 1-to-10													Average Seed for Pods with Seed
				0	-10	-20	-30	-40	-50	-60	-70	-80	-90	-100	-110	-120	-130	
Isolation, Insect-pollination																		
S. no. 1	?	0																
S. no. 2. (1918)	?	17	17	10	7													3.28
S. no. 2. (1919)	?	161	100	0	34	30	16	4	5	4	0	2	1	2	0	2		23.67
L. no. 1	?	65	65	16	47	2												2.96
L. no. 2	?	53	53	3	47	3												4.85
M. no. 2	?	3000	300	14	202	48	8	7	6	1	5	1	1	1	2	3	1	8.91
M. no. 1. (1919) upper 1/3	?	many	71	7	43	12	3	2	1	0	2	0	1					
M. no. 1. (1919) middle 1/3	?	few	31	3	16	3	0	3	2	1	2	0	0	0	0	1		
M. no. 1. (1919) lower 1/3	?	few	40	6	20	3	1	2	2	0	1	1	0	1	2	1		
" total	?	1500	142	16	79	18	4	7	5	1	5	1	1	1	2	2		18.25
Hand-pollination																		
M. no. 1. (1919)	233	134	134	1	28	29	19	20	14	6	5	8	3	0	0	1		24.61
M. 5-1 no. 13	103	0																
M. 3 no. 7	48	8	8	1	5	2												7.00
M. 1 no. 48	35	6	6	0	1	2	2	1										20.33
M. 1 no. 31	33	4	4	0	0	2	1	1										25.00
M. 1 no. 56	60	9	9	0	0	4	3	0	1	1								28.33
M. 5-1 no. 25	32	9	9	0	0	0	2	1	0	3	2	1						50.88
M. 5-1 no. 35	32	5	5	0	0	1	1	0	0	2	0	0	1					49.50
M. 1 no. 30	37	9	9	0	0	0	2	0	2	1	1	0	1	2				59.66
M. 1 no. 57	40	44	44	0	1	7	3	2	7	3	3	12	5	1				63.15
L. 4 no. 2	55	2	2	0	2													6.00
L. 1 no. 66	68	2	2	0	2													6.00
L. 2 no. 18	50	7	7	0	6	1												7.42
L. 1 no. 8	49	22	22	0	11	10	1											8.90
L. 5-5 no. 38	24	6	6	0	2	1	2	1										20.00
S. 5 no. 5	14	4	4	0	1	2	1											14.00

capsules that could be referred definitely to differences in insect visitations or to the constitution of the plants.

Mid-styled Plants Grown in Isolation. A plant of this form (*M no. 2*) was sent to Mr. H. L. Skavlem, who grew it at Carcajou Point, Lake Koshkonong, Wisconsin. Mr. Skavlem states that by the middle of July this plant was "over four feet high with an abundance of bloom which continued for about six weeks." The plant bloomed from July 14, 1918, until the first week in September. There were about 35 well-developed main flower spikes ranging from 3 to 14 inches in length, and the total number of flowers produced was estimated at 8,000. This description would apply as well to any well-developed plant of any of the three forms.

This mid-styled plant was decidedly self-compatible in this isolation test. At least 3,000 capsules matured on it in 1917. A total of 300 capsules were examined and counts were made of the seeds present, with

results as summarized in table 1. The number of seeds per capsule ranged to 121; twenty-eight capsules had more than 30 seeds each, but 202 capsules contained less than 11 seeds per capsule, and 14 contained only rudiments of seeds. The average number of seeds per pod for those that had good seeds was 8.91. In 1918 the self-compatibility of this plant was quite the same as in 1917.

In making the counts of seeds in 300 capsules (1917 crop) of this plant, position was taken into account. Each branch involved in the counts was divided into three sections of equal length, here designated as the lower third, middle third, and upper third. In the lower third of all branches there were 10 capsules with more than 30 seeds each. It is clear that the average number of seeds per capsule was lower toward the top of the branches of this plant, but smaller capsules and fewer seeds per capsule are, as a rule, to be expected toward the apex. Otherwise the plant was rather uniformly highly self-compatible, and capsules with seeds were produced in a considerable proportion of the flowers subjected to open pollination throughout the entire period of bloom.

Another mid-styled plant (*M no. 1*) was grown in my own home garden. This plant made a vigorous growth and produced in 1917 at least 6,000 flowers. At the close of the season it was found that relatively few of the flowers produced pods during the first two thirds of the period of bloom, but that later nearly every flower produced a pod.

In 1918 it was planned to test experimentally the behavior of this plant, and especially to determine if the marked difference in production of fruit involved changes in the innate ability of the plant to produce fruit or indicated merely some difference in insect-pollination. A large, well-developed long-styled plant was planted by its side. The two began blooming only one day apart. The long-styled plant was allowed to bloom, thus affording opportunity for free cross-pollinations by insects between the two, until the 17th of August, when the long-styled plant was cut down. During the time that both were in bloom nearly all flowers that opened on the mid-styled plant developed fine pods, showing that the feeble production of pods during the early part of bloom as observed in the previous year when the plant was in isolation was not due to an impotence of the pistils. During the rest of the season its performance was quite as in the previous year.

In 1919 the long-styled plant was kept cut down so that no flowers were produced by it, and a series of guarded self-pollinations were made on the plant *M no. 1*. Branches were enclosed in glassine paper bags. Whenever pollen from short stamens was used for pollinations, the flowers to be thus pollinated were opened early in the morning and the long stamens were removed, and then pollen from the short stamens was used later in the day when the anthers were dehiscing.

Legitimate cross-pollinations, using pollen of flowers brought in vials from the experimental plots over a mile distant, were made on 22 flowers

during the first 18 days of bloom. The plant was in bloom 55 days. The results obtained for selfing are summarized in table 1 and are shown in more detail in table 2, in which, to facilitate ready comparison, the data are compiled for three periods.

TABLE 2. *Record for controlled pollinations of mid-styled plant no. 1 in summer of 1919*

	1st to 18th Day	19th to 36th Day	37th to 55th Day
Selfed with pollen of long stamens			
Failures—no pods	70	35	9
Pods produced	18	46	70
Seeds per pod—range and average	(0 to 117) av. 34	(2 to 81) av. 32	(3 to 82) av. 32
Selfed with pollen of short stamens			
Failures	33	57	47
Pods	1	1	1
Seeds per pod	37	10	1
Legitimate cross-pollination			
Failures	2		
Pods	20		
Seed-range and average	(12 to 176) av. 98		

Of the 140 flowers hand-pollinated with pollen from short stamens, only three produced pods yielding 1, 10, and 37 seeds respectively, but these may have been due to chance pollination with pollen from the long stamens at the time of their removal or with pollen of other flowers which were enclosed in the same bag. The results indicate that the plant remained decidedly if not completely self-incompatible to pollen of its own short stamens throughout the entire period of bloom.

When pollen of long stamens was used in hand-pollinations, the proportion of pods produced increased as the season advanced, and during the last 18 days of bloom there were but 9 failures out of 79 flowers pollinated. The results show conclusively that the self-compatibility in this plant involves fertilizations from the pollen of long stamens, and also that this compatibility actually increases toward the end of the period of bloom. The increase in compatibility affects, however, the number of pods that are formed rather than the number of seeds in a pod. The average number of seeds in the pods that were produced was almost the same for all periods, but the highest number of seeds in any pod was obtained during the first 18 days of bloom.

The 22 legitimate crosses made during the first 18 days gave 20 pods with seeds ranging in number from 12 to 176, with an average of 98 seeds per capsule. These results show conclusively, as do those of open cross-pollinations during the early part of the period of bloom in the previous year, that the pistils of the plant are highly potent during the period of marked self-incompatibility. The decided change in fruit-production s

hence due to a change in the physiological relations between pistils and the pollen of long stamens. Pollen from both short stamens and long stamens was examined at frequent intervals throughout the entire period of bloom; there was very little abortion, the pollen was successfully germinated in artificial media, and the use of such pollen in legitimate crosses on several dates during the first 30 days of bloom was almost invariably highly effective in pod- and seed-production. There were in this plant no noticeable evidences of impotence of stamens and anthers, such as are to be seen in some plants of this species.

The branches left to open-pollination produced pods quite as in the year 1917. Several of the main branches were selected and divided into thirds, and seeds in all pods in the lower two thirds were counted; then an equal number of pods from the many pods in the uppermost third were taken at random for counts, the entire number, 142, being as near the number of pods secured from the selfing by hand in which the pollen of long stamens was used as was possible. As shown in table 1, the range for number of seeds per pod and the average were higher for the lower two thirds than in the last third, showing that the change in compatibility involves number of pods formed rather than number of seeds per pod. Comparison shows that the controlled pollinations in which pollen from long stamens was used were somewhat more successful than free open-pollinations, when judged by the average number of seeds produced.

A third mid-styled plant (*M no. 3*) was grown in isolation in the New York Botanical Garden at a distance of about one mile from the location of the plant *M no. 1*. This plant proved to be decidedly less self-compatible to open self-pollination than were *M no. 1* and *M no. 2*, but, as in *M no. 2*, there was quite the same proportion of pods produced throughout the entire season of bloom, no change in compatibility being evident as observed in the plant *M no. 1*.

Summary. In these tests seeds were obtained to illegitimate self-pollination of plants of all three forms. Mid-styled plants were most highly self-compatible in respect to the number of pods produced. The pods found were distributed irregularly over the entire period of bloom except for one plant (*M no. 1*), in which it was found that there was an actual change in the degree of self-compatibility to pollen from its own stamens. The results from year to year have been very uniform for all plants, except for the plant *S no. 2*. Its feeble production of pods in 1918, followed by the production of 161 pods in 1919, suggests that the plant is rather strongly self-compatible provided insects make the proper pollinations. It may readily be conceded that the kinds of insects that can most efficiently self-pollinate long-styled and mid-styled plants are not the ones which best self the short-styled plants.

Self-pollination does not appear to be uniformly as efficient in seed-production as are certain legitimate cross-pollinations, at least for the one

mid-styled plant *M no. 1* (see table 2). Of the 22 flowers on it that were crossed during the first 18 days of bloom there were only two failures, the highest number of seeds for a capsule being 176 and the average 98. Whether such pollination would be more effective than selfing during the last part of the period of bloom was not tested.

Self-compatibility Tests by the Bagging Method

A total of about 600 plants have been grown in pedigreed cultures from seed. A considerable number of these have been tested for self-compatibility in the following manner: branches were enclosed in glassine paper bags, and pollinations of flowers opening within were made from day to day. Long-styled plants and short-styled plants were selfed by using the pollen from mid-length stamens, and the mid-styled by use of the pollen of long stamens. In making pollinations, stamens with dehiscing anthers were removed with sterile tweezers and brushed on pistils, leaving an abundance of pollen. In cases of pseudo-proterogyny the fully protruding pistils of partially opened flowers were likewise treated along with pistils of more mature flowers. It appears that in the decidedly pseudo-proterogynous flowers the pistils protrude long before they are receptive to any kind of fertilization, and that highest seed production in compatible fertilizations occurs when pollination is made at or about the time that petals open and pollen is shed. On plants two or more years old, a total of as many as 500 or more flowers were often thus pollinated. On plants in the first year of growth from seed the number thus selfed was often much less.

These tests are undoubtedly less adequate than tests in isolation for revealing feeble grades of self-compatibility and in showing such changes in self-compatibility as are seen in the plant *M no. 1*, but hand-pollinations make certain that pollen in abundance is applied to the stigmas at the time when they are judged to be most receptive.

The general results summarized for each form without reference to lines of descent are as follows:

	Fully Self-incompatible	Feebly Self-compatible	Medium Self-compatible	Highly Self-compatible
Mid-styled plants.....	64	20	21	7
Long-styled plants.....	83	14	0	0
Short-styled plants....	22	1	0	0

An attempt has been made to grade the plants according to whether the self-compatibility is feeble, medium, or strong, the judgment being based on the proportion of selfed flowers that gave pods and the number of seeds produced. Results typical for various grades are given in table 1. The tests made show that many plants of the species are without doubt entirely self-incompatible (*M 5–1 no. 13* in table 1 for example), and that others are

highly self-compatible (*M 1 no. 57*), with various intervening grades the grouping of which is neither definite nor accurate.

In these tests mid-styled plants have been more highly self-compatible than plants of the other forms. This is true both in relative numbers that produce fruit to selfing, and in the range to higher grades of fertility. On one plant, *M 1 no. 57*, every flower that was selfed produced a pod, and four other pods were produced in other flowers that spontaneously selfed while enclosed in a glassine bag.

Of the 97 long-styled plants tested in hand-pollinations, only 14 produced pods, and not one gave over 30 seeds in any pod. In all of these the self-compatibility was apparently of a weak grade.

Twenty-three short-styled plants were tested, and only one produced seeds.

The results obtained in the controlled self-pollinations with these plants agree in general with those obtained in isolation tests. A rather large proportion of mid-styled plants are self-compatible in some degree, and nearly half of the plants of this form produced pods containing viable seeds to selfing, and a few were highly self-compatible. There has been no difficulty in finding mid-styled plants to use as parents of self-fertilized lines of progeny. Relatively few long-styled plants produced pods to selfing, and in all such plants the self-compatibility was feeble, few pods being produced and these having few good seeds. Short-styled plants have as a class been decidedly self-incompatible, and of the seedlings tested only one has produced seeds to selfing. The high seed-production seen in the plant *S no. 1* in 1919 was not duplicated by the plant in 1918 nor in 1920 and 1921. There has not been opportunity to test this plant by controlled hand-pollinations as the plants grown from seed have been tested.

Summary

1. Many plants of *Lythrum Salicaria* are capable of producing capsules and viable seeds to illegitimate self-pollination brought about either by controlled hand-pollination or by insect-pollination in the field. The capacity for self-fertilization still lingers strongly in the species.

2. The proportion of self-compatible plants is greatest in the mid-styled plants, in which also the highest grades of self-compatibility are to be seen. Long-styled plants are, as a class, less self-compatible, and the short-styled plants are still less so. The three forms appear to differ in the capacity for self-compatibility.

3. There are wide variations in the degree of self-compatibility. In the most highly self-compatible form, the mid-styled, there are all gradations between complete self-incompatibility and the highest grade of self-compatibility.

4. The variations in the physiological condition of the sex organs, as

exhibited in selfing, suggests that wide variations may likewise be expected for crossings even for those that are legitimate.

5. One noticeable case of end-bloom self-compatibility was found. This was in a mid-styled plant and involved only fertilizations from pollen of the set of long stamens.

6. The physiological relations of the sex organs in plants of this trimorphic species exhibit quite the same range of variations as are seen in many homomorphic species.

Conclusion

For the species *Lythrum Salicaria* the evidence of wide variation in the degree of self-incompatibility is definite. The physiological differentiations of the sex organs are incompletely correlated with the apparent structural adaptations for cross-pollination; they are not fixed, constant, and fully achieved either in expression or in heredity, but are fluctuating and intergrading. They still present opportunity for further selection either toward greater or toward less restriction of fertilization.

The persistence of self-compatibility in various degrees of expression, and the apparent difference in respect to self-compatibility seen among the various forms, present strong evidence that self-compatibility was the antecedent condition in the species out of which the present complex of sex relations is still evolving, just as the sets of styles and stamens of different lengths have been developed out of an original homomorphic species.

New York Botanical Garden

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CHROMOSOME BEHAVIOR IN *ACER PLATANOIDES* L.

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That chromosomes may be observed as definite bodies at nearly all stages in the life of a cell has been shown by various workers. Rosenberg (1904) and Overton (1905) were among the first to direct attention to the individual chromosomes in the resting nuclei in plant cells as definite chromatin bodies. In addition, Overton (1909) has figured these chromatin masses, "prochromosomes" as he termed them, in the various stages of the reduction division in the pollen mother cells of Thalictrum, Calycanthus, and Richardia. He maintains that these prochromosomes are in pairs in the resting nucleus of the mother cell; that in synapsis a continuous bivalent spirem exists in which the paired prochromosomes are arranged side by side at more or less regular intervals, and that they remain thus associated in parallel pairs until they separate at metaphase. Evidently these observations and conclusions of Overton are in support of the Grégoire school which maintains that there is a side-by-side pairing of the chromosomes in synapsis.

Digby (1919) has brought forward some corroborative evidence in support of the view of Farmer and his followers that the splitting of the spirem preceding synapsis is the same as the splitting in the telophases of the sporogenous mitosis, and that there is an end-to-end pairing of the chromosomes in synapsis rather than a side-by-side pairing. The difference between these two views of how the chromosomes pair appears to be due largely to differences in interpretation of stages associated with synapsis. Evidence which has a direct bearing upon this problem has been found in the study of the heterotypic division of the pollen mother cells of the Norway maple, *Acer platanoides*; in this plant the behavior of the individual chromosomes may be followed with considerable clearness through synapsis. Cardiff studied this species, but his findings differ in several particulars from those of the writer, so that a reconsideration of this form seems justified.

METHODS

In the preparation of the material for this study, the flower buds of *Acer platanoides* were collected in the early spring when the buds first began to enlarge. After removing the scales, the buds were dipped in 50 percent alcohol and then fixed in either Flemming's medium solution or in Bouin's solution. Dipping in alcohol caused the flower cluster to sink at once when placed in the fixing fluid and resulted in more uniform fixation of the

material. Bouin's solution gave the better results in fixation, causing less shrinkage of the cytoplasm and less clumping of the chromosomes. The best results in staining were obtained by using the safranin, gentian violet, and orange G combination. The sections were first bleached for twelve hours in full-strength commercial hydrogen peroxide; following this they were stained one minute in a saturated, 50-percent alcoholic solution of safranin; five minutes in a similar solution of gentian violet; a few seconds in an aqueous solution of orange G; the slide was then flooded with absolute alcohol followed with clove oil, which was allowed to remain until the stains had been extracted so as to give the desired differentiation. This method gave a large number of excellent preparations showing marked differentiation between the nucleolus, linin, and chromatin contents of the cell. Heidenhain's iron-alum haematoxylin stain was used as a supplementary one, but it did not give the satisfactory differentiation of the triple stain.

Observations

Attention was given primarily to the nuclear changes in the pollen mother cell during the stages of its first division. The somatic cells of the flower stalk and of the roots were examined as well as several megaspore mother cells. The pollen mother cells were exceptionally good for study in that each flower bud frequently showed several stages of development, a single loculus of an anther often including a progressive series of stages from one end to the other.

In the earliest stage to be found in the pollen mother cell, the chromatin-staining material exists as several well-defined bodies, varying somewhat in size, which are distributed about the periphery of the nucleus; a few of these bodies, however, appear to be scattered through the nucleus, and some are usually lying against the nucleolus (Pl. XXXI, fig. 1). Not infrequently two chromatin bodies are found lying near each other; because of the similarity in size and position one is led to believe that they belong together as a pair. The distance between the individuals of a pair varies; sometimes they are nearly in contact, while in other pairs they are separated by a space at least equal to their own width. Some of the bodies do not appear at first to be paired, yet in view of their subsequent behavior it is not difficult to associate practically each one with a nearby mate. It is not easy when one sees so many conspicuous cases of pairing in the nuclei of this stage to believe that these are merely chance positions, as interpreted by Mottier in *Acer negundo*. The number of these chromatin bodies is probably twenty-six, as determined by an examination of numerous complete nuclei.

The linin occurs as very fine, faintly stained threads, usually forming an irregular netlike structure to which the chromatin bodies appear to be attached. Occasionally enlarged nodes will be observed on the linin where two threads cross or come together; these nodes are distinguished from the

chromatin bodies by their faint staining reaction and their irregularity of outline.

Attached to the nucleolus is usually one, rarely two, bud-like structures which take the safranin stain like the nucleolus; sometimes they are found disconnected from the nucleolus. They vary somewhat in size but are usually somewhat larger than the chromatin bodies, from which they are further distinguished because of their reaction to stains and because of their more globular shape (fig. 2).

The first evidence of growth on the part of the mother cell is an increase in size of the nucleus as well as of the entire cell. The linin stains more readily, and many of the chromatin bodies are more conspicuously arranged in pairs. At no stage could I be sure that the individuals of a pair are connected by linin. The cytoplasm becomes somewhat less dense as the cell enlarges, but the changes are so slight that one would not be justified in using them as criteria for determining the stages of development of the nucleus as did Cardiff in his study of this species.

The next apparent change is a more conspicuous linin which becomes pulled away from the periphery of the nucleus in several places and collected to form the synaptic knot (fig. 4); usually, even at the most contracted phase of synapsis, there are a few free threads extending outside of the knot, some of which reach to the nuclear membrane (fig. 5). There is no conspicuous paralleling of the threads during the pre-synaptic stages nor in synapsis; only occasionally will two threads be seen parallel to each other. The chromatin bodies remain distinct throughout the early stages of synapsis; as the linin becomes more contracted into a knot, the individuals of a pair come closer together until they are in contact with each other. Throughout these stages there are always some chromatin bodies lying against the nucleolus, and frequently a group of four will be found rather close together.

At about the period of maximum contraction of the linin the chromatin bodies appear to lengthen out; the individual members of a pair seem to flow out in opposite directions, indicating an end-to-end union of the two; in this manner short, thick threads are formed which take the chromatin stain (figs. 7–9). In a single nucleus some of the chromatin material may be seen as definite paired bodies, while some appears as short, thick threads which fade into the less deeply stained linin. In a few cases one or more chromatin threads were found outside of the knot with only one end entering it; upon these threads were darker-stained portions indicating the position of the pair of chromatin bodies. No evidence was found to indicate that two fine parallel threads unite to form a single large one.

The synaptic knot becomes more and more converted into thick threads by the flowing out of the chromatin material until there appear to be several of these thick threads, more or less massed, with some free ends extending out from the knot (fig. 11). It is possible that these threads are

all joined together into a single long, tangled one, but after an examination of numerous cells of this stage, such a conclusion seems hardly warranted. These thickened threads soon become less tangled, usually with one end extending into a clump at one side of the nucleolus (Pl. XXXII, fig. 13); this stage resembles that figured by some workers as the second contraction stage. Commonly one or more individual threads do not extend into the clump. Many of the threads at about this stage show a lighter longitudinal area indicating a longitudinal splitting; the splitting becomes so nearly complete in some cases as to separate the two halves of the thread for at least part of its length. These threads often contain as many as four or five deeper-staining places somewhat varied in size; on the split threads these appear as paired, equal-sized bodies (fig. 13). At about this stage the cytoplasm becomes drawn away from the cell wall and more condensed about the nucleus.

The next apparent change in the cell is in the general distribution of the threads throughout the nucleus; many of the threads are bent or looped (fig. 14). As development proceeds, the threads become shorter, usually more ragged, and several of them soon become shaped like a *C*, a *U*, or an *O*; at this stage the split has disappeared (figs. 15–17). One cannot be certain that each one of the threads folds over on itself; in fact, after studying numerous nuclei of this stage and after considering the subsequent stages in the separation of the individuals of a pair, I am of the opinion that some threads do fold over and some do not. There is no direct evidence that the bend in the thread occurs where the two chromatin bodies of a pair unite, but it is reasonable to suppose that such is the case. These bent threads become shorter, and some of them at least segment at the bend; contraction and condensation of the chromosomes continue while the membrane of the nucleus disappears (figs. 18, 19). Some of the threads become condensed into chromosomes before others; this fact may be associated possibly with the difference in size of the chromosomes which one observes at metaphase. This irregularity, or variation, in development is also evident as the chromosomes are separated at metaphase, some individuals of a pair being entirely separated while others are intact. As the members of a pair are being separated, some of the elongated ones show rather clearly that they are lying side by side in the spindle. There is no evidence of splitting of the individual members of the bivalents during these stages. When the nuclear membrane disappears, the chromosomes become collected about the nucleolus, which in turn becomes elongated and irregular, gradually disappearing as the chromosomes become arranged on the spindle.

As the univalent chromosomes move toward the poles they appear to become more condensed, forming short, thick lumps; there is some indication of a splitting of the univalents during this stage, but the evidence is not convincing. At the poles the chromosomes retain their individuality; they become somewhat larger, irregular in shape, and some, at least, have

one or more threads attached to them (fig. 21). As the daughter nuclei increase in size, one or more nucleolar bodies appear in connection with some of the chromosomes. In the resting stage of the nucleus the chromosomes become somewhat more condensed, and there is usually but a single nucleolus present (figs. 22 *A*, 22 *B*). This resting stage appears to be of short duration. The chromosomes become more angular during the succeeding prophase stages and appear to be split about the time that they are drawn toward the equatorial plate; several of them are in contact with the nucleolus, which in turn becomes irregular and extended. In some cases the two spindles are arranged in the same plane, but more usually they are at right angles to each other. The further stages of division of the daughter nuclei appear to be like those of the heterotypic division. The tetrad or granddaughter nuclei show clearly the thirteen chromosomes scattered about in the nucleus (fig. 23).

In the resting stage of the vegetative cells the chromatin occurs as definite chromatin-staining bodies; there is no conspicuous pairing of these bodies in this stage, but when the nucleus is undergoing the late prophase stages in division, the association of chromosomes of similar shape and size does become apparent (fig. 25), although they were never found joined together; this same condition of pairing was observed in the metaphase stage (fig. 24).

In the megaspore-mother-cell nucleus, several prophase stages were observed including synapsis, but all presented the same phenomena as those observed in similar stages of the pollen mother cell.

Discussion

Cardiff in his work on *Acer platanoides* called attention to the chromatin in the resting nucleus of the mother cell as collected in small bodies at the periphery of the nucleus, but he did not consider their number. Although one cannot be entirely certain, yet I am convinced that the number corresponds to the number of chromosomes observed in the division stages of the vegetative cells. These chromatin masses are doubtless a phase in the life history of the chromosomes, and correspond to the prochromosomes of Overton. In my earlier work on *Acer negundo* similar chromatin bodies were observed, but no attention was given to the number present; a reexamination of these stages as well as of later ones has led me to conclude that the phenomena of chromosome-formation in the two maples are essentially the same and that certain stages in the preceding work were misinterpreted.

The bud-like structures on the nucleolus were noted by Cardiff and figured both by myself and by Mottier in *Acer negundo*. There is evidence that these break off from the nucleolus during the growth period of the cell, since occasionally one or more are found lying free in the nucleus; this fact may be determined in nuclei well stained with the triple stain, in which the nucleolus and these buds stain with the safranin and the chromatin bodies

stain with the gentian-violet. These free buds disappear as development proceeds. As to the significance of this behavior there is no direct evidence; it may possibly be an early fragmentation of the nucleolus, or it may be associated with chromatin anabolism; the latter is perhaps the more probable in view of the subsequent close association of nucleolus and chromatin in the late prophase stages of the mother cell.

The significance of the synapsis stage is a topic that has been frequently discussed; many students of the cell have considered it to be the time of pairing of the paternal and maternal elements. Overton, however, says that in the plants with which he worked the prochromosomes are already paired at the stages of greatest chromatic distribution of the mother cell, and that the pairing may possibly occur in the telophases of the last pre-meiotic division. In these cases the individuals of a pair are very closely associated, in fact, so much so that in his earlier work the author considered each pair a single body. In *Acer platanoides* there may be a general grouping of the chromatin bodies into pairs even in the resting stages of the nucleus, but their close association does not take place until synapsis. This evidence is in accord with the belief that synapsis is a significant stage in the heterotypic division.

The manner in which the members of a pair of the chromatin bodies become joined together is shown to be an end-to-end arrangement; this fact is verified by the stages immediately following their union when the chromatin threads are formed by the flowing out of the chromatin in opposite directions from the paired chromatin bodies. There certainly is no evidence that I can find to support Cardiff's more or less diagrammatic figures of a side-by-side pairing before synapsis; his figures 5 to 13, inclusive, which he interprets as pre-synaptic, are more suggestive of post-synaptic stages; he calls attention to the fact that the threads are arranged in pairs and that most of the pairs seem to be in contact with the nucleolus, or very near it; this is a condition which I find in post-synapsis.

The interpretation of the nature of the pre-synaptic spirem has been the principal factor in the divergence of conclusions reached by the parasynaptic and telosynaptic schools. In forms like *Acer platanoides* and *Acer negundo*, as reported by Mottier, in which a pre-synaptic spirem does not occur, and in which the chromatin bodies are not split, this factor of whether the spirem is split or double is removed and the evidence for the manner of pairing becomes more conclusive.

The chromatic spirem in *Acer platanoides* is formed during the synaptic contraction by the flowing out of the chromatin-staining material along the linin in much the same manner as found by Overton in Thalictrum. Mottier apparently did not determine the behavior of the chromatin masses in *Acer negundo* during the synaptic stages, but he states that the spirem is formed during this stage.

The formation of the bivalent chromosomes from the thick chromatin

threads suggests nothing unusual, unless it be that some of the threads fold on themselves and some do not, as the examination of several cells has led me to suspect.

The direct origin of the chromosomes in the homoeotypic division from the chromatin masses in the daughter nuclei and the subsequent behavior of these chromosomes in the formation of the granddaughter nuclei are strong evidences in support of the theory of the permanence of the individual chromosomes.

Summary

1. Chromatin masses corresponding in number to the chromosomes at the time of cell division are present in the various stages of the vegetative cells and of the reproductive cells in *Acer platanoides*.

2. These chromatin masses may be followed through synapsis, in which stage they become closely paired and unite end to end.

3. The chromatin threads are formed by a flowing out of the chromatin material from the chromatin masses.

4. The chromosomes do not lose their individuality in passing through the telophase stages to the resting stage of the nucleus.

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EXPLANATION OF PLATES

All figures were made with the aid of a camera lucida with Spencer 1.5-mm. objective and 10x eyepiece. Drawings magnified about 2900 diameters.

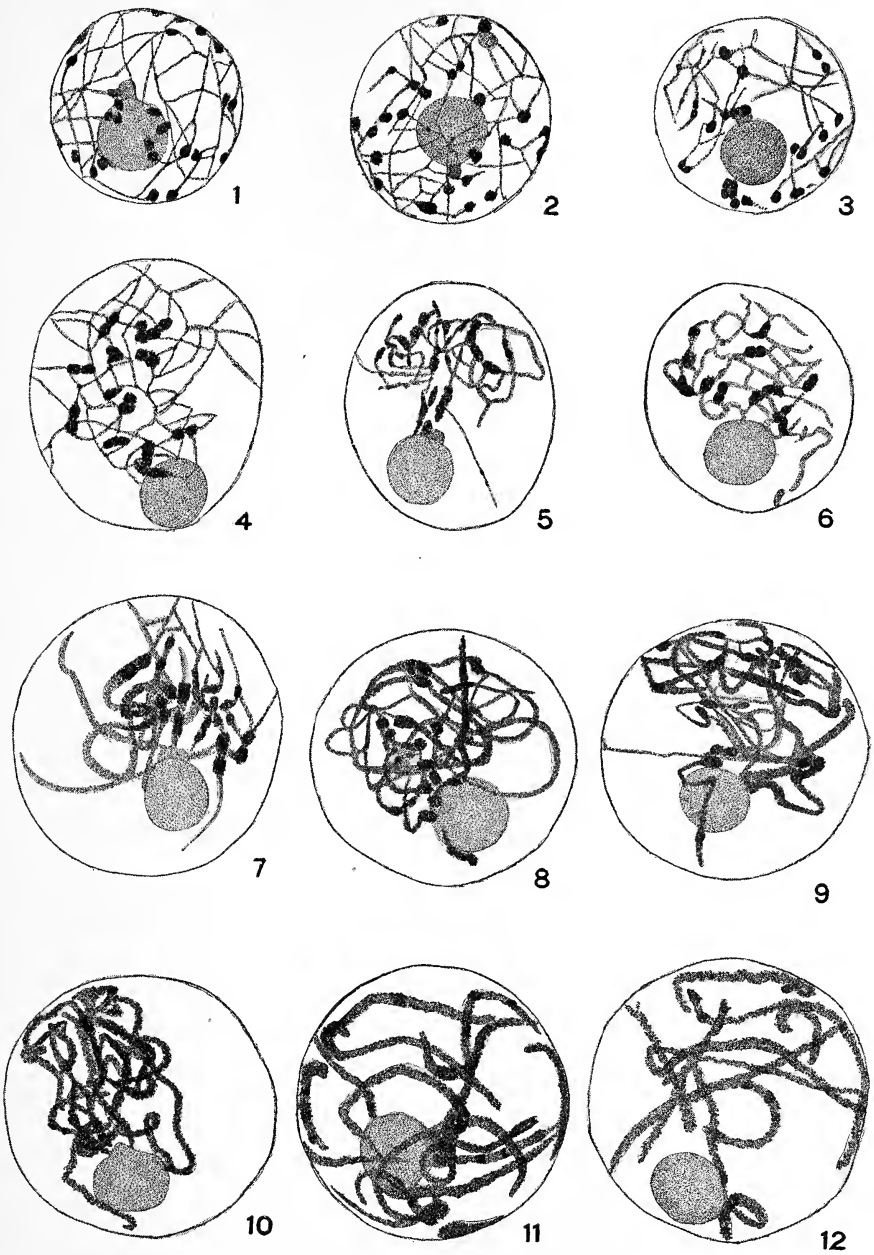
Plate XXXI

Fig. 1. Resting nucleus of the pollen mother cell, showing chromatin bodies, net-like linin, and bud on nucleolus.

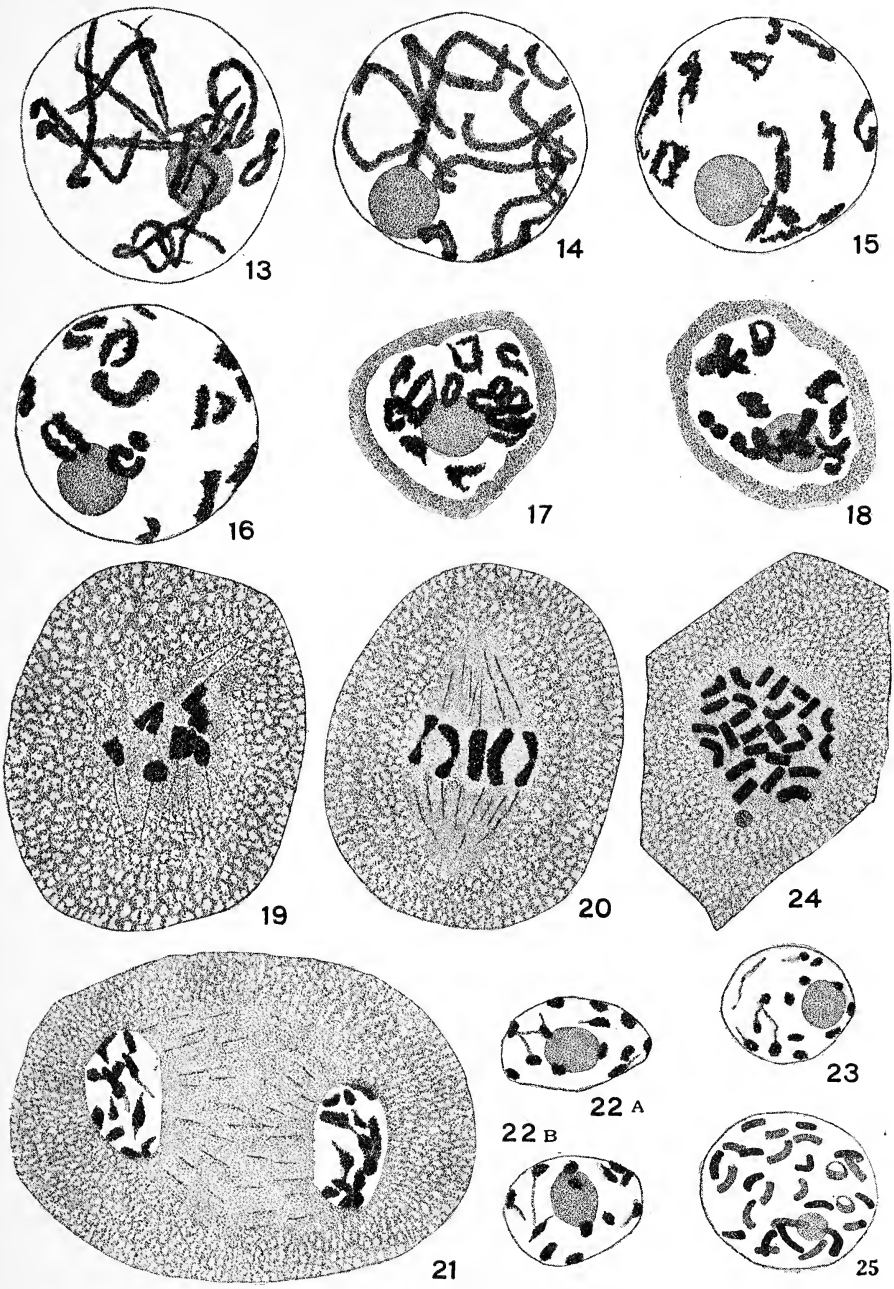
Fig. 2. Early growth period of the pollen-mother-cell nucleus, with a bud freed from the nucleolus.

Fig. 3. Early growth period of cell, showing conspicuous pairing of chromatin bodies.

Fig. 4. Early stage in synapsis, the linin becoming drawn into a mass and the chromatin bodies often joined together end to end in pairs.



DARLING: CHROMOSOME BEHAVIOR IN ACER



DARLING: CHROMOSOME BEHAVIOR IN ACER

FIGS. 5, 6. Portions of nuclei during early synapsis, the linin threads becoming thicker and the chromatin bodies more closely joined in pairs.

FIGS. 7, 8, 9. Stages in synapsis in which the chromatin becomes spread out from each pair of chromatin bodies to form the thick chromatin threads.

FIG. 10. Late synapsis, in which the chromatin bodies lose their identity in forming the chromatin threads.

FIG. 11. A stage in which the threads are becoming loosened from the synaptic knot.

FIG. 12. A portion of a nucleus in an early post-synapsis stage.

PLATE XXXII

FIG. 13. A post-synapsis stage, showing the split threads and the split granules on the threads.

FIG. 14. The chromatin threads, usually bent, are beginning to shorten.

FIGS. 15, 16. Diakinesis; some of the threads appear to be folded to form loops, some do not.

FIGS. 17, 18. The nuclear membrane disappearing and the chromosomes becoming more condensed and clustered about the nucleolus.

FIG. 19. A portion of a mother cell with spindle fibers becoming attached to chromosomes; some long chromosomes show the side-by-side pairing as a result of the folding of the chromatin thread.

FIG. 20. A portion of a mother cell in late metaphase; some bivalents are separated more than others.

FIG. 21. Early telophase; the chromosomes retaining their individuality.

FIGS. 22 *A*, 22 *B*. Resting daughter nuclei of a single cell, showing the thirteen chromosomes in each nucleus.

FIG. 23. A tetrad or granddaughter nucleus in the resting stage, showing the thirteen chromosomes.

FIG. 24. A polar view of the metaphase stage of a vegetative cell from the flower stalk, showing a general paired condition of the chromosomes and a discarded portion of the nucleolus.

FIG. 25. A late prophase stage of a vegetative cell from the flower stalk, showing a general pairing of the chromosomes.

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THE PHYSIOLOGY OF INCOMPATIBILITIES[1]

A. B. STOUT

In general survey, physiological incompatibilities in fertilizations include two groups of phenomena:

1st. There are the very general and characteristic failures of cross-fertilization *between* different species, long considered as the most adequate evidence of specific distinction; and

2d. There are those physiological limitations to free and general fertilization *within* species exhibited best in the failure of certain homomorphic hermaphrodites to self-fertilize, but also in the cross-incompatibilities among seed-grown individuals of the same species or race.

Certain aspects of the physiology of these incompatibilities are clear. They exist and are in operation when the sex organs and sex elements are in a condition for proper fertilization; the elements do not function in certain relations but do in others; fertilization is discriminative.

It is not, therefore, a question of what brings the spores or gametes to ripening, or of the mere production of those egg or stylar secretions or chemotactic influences which make fertilization possible. It is rather a question of a very special kind of development or physiological condition which discriminates between fertilizations when they are otherwise possible.

Inter-specific incompatibilities are very generally considered to involve species specificity. They are expressed in the interaction between egg secretions and sperms, in mechanical and chemical resistance of the cortical layers of eggs to the entrance of sperms, in the extrusion of sperm chromatin after fertilization, in the death of the heterogenetic hybrid, or in the sexual impotence of such hybrids. In all these ranges of expression the incompatibilities appear to be deep-seated and inherent in the physical and chemical differences in the organization of species.

Turning now to intra-specific incompatibilities, there is apparently a very different physical basis. Here there is self-incompatibility involving the germ cells of a single individual. Here also there is cross-incompatibility between individuals of the same parentage. Such cases are frequent among homomorphic hermaphrodites. They are so widely distributed in the families of flowering plants that it would seem that there must be some

[1] Read in the symposium on "Sterility in Plants," at the joint meeting of Section G of the American Association for the Advancement of Science, the Botanical Society of America, and the American Phytopathological Society, at Cambridge, December 27, 1922.

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fundamental principle operating in their origin and development. In these higher plants there is the development of a nearly naked egg (free of a decided membrane) imbedded in the tissue of the ovule, and a highly specialized male gametophyte—the pollen tube—with its various growth relations in the style. While fertilization in these cases involves a series of physical and chemical relations, it is fundamentally an egg-and-sperm reaction.

It is to be noted that studies of the physiology of pollen germination have failed to reveal a specificity that is comparable or related to the compatibility or incompatibility found in homomorphic species. It has, however, long been known that in cases of self-incompatibility the pollen tubes often make feeble growth in the style, and more recently it has been shown that their rate of growth is not accelerated as the eggs are approached. This condition apparently persists when incompatible pollen is mixed with compatible pollen and the tubes of both grow side by side in the style. This indicates that the reactions are decidedly discriminative and suggests that they involve reactions of the pollen tubes to secretions of the eggs. There is, however, evidence that in many grades of self-incompatibility the injurious effects may be exhibited after what is apparently a successful fertilization.

One aspect of the development of self-incompatibility in the hermaphrodite is clear. Every such individual is itself the result of a compatible fertilization in which two cells fused, and the two elements of the resulting diploid organization show themselves compatible throughout the somatic life of the individual; but the two kinds of sex elements produced by it are incompatible, and this is of course irrespective of their germ-plasm complex. The incompatibility arises along with sex differentiation, which in seed plants begins with the development of pistils and stamens and is independent of any readjustments of the germ plasm in the reduction divisions.

The biogenetic nature of the development of self-incompatibilities is further indicated by the wide variations which exist in their expression in individuals. Between the extreme or alternative conditions there are many intergradations, and the extreme conditions are reversible in a progeny. This is the general rule of behavior in such pedigreed cultures as have been critically tested in this particular. Cross-incompatibilities exhibit quite the same ranges of expression, and here reciprocals may give directly opposite results.

But there is also conclusive evidence in certain species of cyclic changes in the self-compatibility of an individual. These are best seen in plants which have a somewhat extended and continuous period of bloom. In some species there is self-compatibility at the end of the period of bloom; in others the climax of self-compatibility is at the mid-period of the bloom, and for certain perennials there is some evidence of changes from year to year in relation to the age of the plant. It is to be noted that a mid-period

self-compatibility is preceded and followed by self-incompatibility. There is alternative expression of extreme grades of compatibility and incompatibility in the series of flowers produced in succession on the same branch. The functions of fertilization are in such cases operating in a cycle of intensities.

It may here be reported that, in the species *Brassica pekinensis*, self-incompatibility of a plant as a whole or of a family of plants may be decidedly changed by a cultural treatment which reduces vegetative vigor. In a family of this species grown for three generations, less than 10 percent of the total of 326 plants were highly self-compatible and there was no hereditary effect of selection for self-compatibility. When a generation of this family was grown in small pots with decided reduction of vegetative vigor, of a total of 1,128 plants there were 734 (65%) that were highly self-compatible, and only 22 (less than 2%) were self-incompatible. Furthermore, a large proportion of the former were self-compatible in the earliest flowers that opened. The family was decidedly changed in regard to the number of plants that were self-compatible, and in the individuals the characteristic cycle was altered. Such results, together with the other behavior noted above, indicate that there is a direct and very decided physiological correlation between vegetative vigor and the functional properties of the organs concerned with fertilization.

This is at least suggestive that the physiological conditions which restrict and limit indiscriminate fertilization within species are not only subject to internal regulation, but that in some cases at least they are correlated with changes in vegetative vigor.

The situation gives hope that the cyclic expression of sexual affinities and the development of extremes of compatibility may be so regulated experimentally that the specific biogenetic factors and conditions operating in this highly specialized differentiation may be determined.

New York Botanical Garden

POLLEN- AND SEED-STERILITY IN HYBRIDS[1]

Bradley Moore Davis

Sterility of hybrids in various forms and degrees is a phenomenon so frequently presented to the plant breeder and geneticist that in some form and in some degree it is rather to be expected. The first problem in its study demands a critical examination to determine in the life history the place of those conditions that bring about the sterility in question. In earlier days the gametes were generally expected to carry the blame of failure to reproduce the line. More recent studies have shown that responsibilities for sterility cannot be so easily placed.

Sterility, as expressed by varying proportions of abortive pollen and abortive ovules, is very common, and since it is easily recognized this manifestation of sterility has received the greatest share of attention. It is expressed by failure of the pollen grain to attain full size, the structure shriveling and usually losing its protoplasmic contents. In a like manner, the megaspore or embryo sac does not reach a normal development in the ovule. Such behavior results in failure to produce gametes, and cytological studies, as far as they have gone, indicate that this form of sterility, at least frequently, has its causation in irregularities of the reduction divisions which immediately precede the differentiation of micro- and megaspores.

During these mitoses spindles may not be normal in form, chromosomes may be distributed in varying and irregular numbers, and the preparations for the reduction divisions may show abnormalities. Such phenomena clearly indicate a breakdown in the mechanism of nuclear division at this critical stage in the life history. It seems reasonable to assume in these cases that the hybrid must carry a germ plasm the structural elements of which can not conduct themselves in the orderly manner so characteristic of meiosis. Speculation on the reasons for the obvious breakdown of the cell and nuclear mechanism at this point in the life history would lead us too far afield for the purposes of this paper. It seems clear, however, that the causes lie in the heterozygous nature of the germ plasm, since we do not find this form of sterility in pure material. Abnormalities of chromosome distribution are clearly invited when the two sets are of different genetical constitution, since irregularities of segregation are rendered much more likely.

It should not be assumed, however, that the presence of abortive pollen and abortive ovules is proof positive that the parent plant is hybrid, although

[1] Read in the symposium on "Sterility in Plants," at the joint meeting of Section G of the American Association for the Advancement of Science, the Botanical Society of America, and the American Phytopathological Society, at Cambridge, December 27, 1922.

any plant showing high proportions of shriveled pollen may very justly be an object of suspicion. There is a form of pollen sterility due to malnutrition, and this condition may be brought on experimentally by operations seriously affecting the vegetative activities of a plant, as, for example, stripping the leaves from stems. In this connection should be mentioned a form of abortion presented in heterosporous plants when one or more megaspores, perhaps through more favorable position or the good fortune of a better start, are able to develop at the expense of their neighbors which, giving up their substance, are sacrificed for the good of those megaspores that survive. This is fundamentally a form of sterility due to malnutrition, but it is not of course peculiar to hybrids.

Sterility which results from failure to develop gametes is one thing, and sterility due to the inability of gametes to unite is quite another. The latter form of sterility is much more difficult of study in plant material than in animal because the fusion of gametes in higher plants takes place in structures and tissues difficult of examination. Zoölogists recognize the phenomenon and it may be expected to be present in plants, but forms most profitable for study seem rather more likely to be found in groups of the thallophytes than among the higher plants. Sterility of this character need not be due to particular physical or chemical conditions that prevent the union of gamete protoplasts. Pollen grains may not find the secretions of the stigma favorable for their germination. There is probably a very large amount of sterility which results from the inability or slowness of some pollen tubes to penetrate certain lengths of style. We know something of this matter through the studies of East on Nicotiana, of D. F. Jones with pollen mixtures in maize, and from observations of Buchholz and Blakeslee on pollen-tube growth in Datura. It is probably a factor of importance in determining some of the results of Oenothera breeding.

Zygotic sterility is very common among plant hybrids. It means that the zygote is either unable to develop at all or that it produces an embryo which dies early in the production of a seed-like structure. In either case this form of sterility may be suspected from the presence of shriveled ovules or shriveled seed-like structures of various sizes, but generally smaller than normal seeds. Sometimes there may be structures as large as seeds and externally like them but without embryos. Zygotic sterility is therefore conveniently recognized by seed sterility, only care must be taken to make sure that seeds are really sterile because of internal conditions and not merely slow of germination. In plant genetics it is necessary to know these facts before conclusions can be drawn on the significance of so-called "percentages of germination." Conditions must be arranged to force seeds to complete germination, and examinations of the residue of ungerminated seed-like structures must be made to make sure that germination is really complete.

Of course it is impossible to draw a fast line between the embryo which dies in the seed and the embryo which comes forth a weakling unable to

live long as a seedling. The geneticist working in certain groups knows this latter type of seedling very well. Oenothera material is full of illustrations. One type is that of a seedling which expands green cotyledons but the hypocotyl is totally unable to develop a root. Other forms are delicate, frequently etiolated seedlings which live only a few days even when nursed along with most particular attention to their needs of suitable soil, careful watering, and cooler temperatures. Perhaps some of these seedlings are accidents of development, but it is clear from many studies that there are large groups in which the inability to develop rests on a genetical basis and is really the expression of a degree and form of sterility.

Thus, from forms of zygotic sterility expressed by abortive embryos in seed-like structures, we pass insensibly to conditions illustrated by classes of weak seedlings which make a start only to die sooner or later. This is truly a form of sterility when the behavior is due to the germinal constitution of the seedling, and it is impossible to draw lines sharply in the wide range expressed by the various degrees of impotence. Examples of this form of sterility are well known to every plant geneticist who follows carefully the fate of seedlings from hybrid material when germination is complete. It not infrequently happens that large groups of etiolated seedlings appear, or weak dwarfs of various forms, or plants which make considerable growth but fail to mature. Most of these products die early even with the best of care; very few will live under the conditions of the open garden.

In the foregoing sketch I have endeavored to make clear the fact that sterility in plants is expressed in a number of very different forms and may operate at several points in the life history. The fact that the life history of plants is made up of two generations, gametophyte and sporophyte, with the three critical periods of chromosome segregation, fertilization, and embryo development frequently associated with involved physiological and histological conditions adds greatly to the complications of observation and interpretation and makes the study of sterility in plants more difficult than that of animals.

With so many forms of sterility known and expressed in such various ways, the specific causes must be very numerous. Some of them may be relatively simple, as when pollen fails to function because of delay in germination or slowness of tube growth. Even a breakdown at the time of the reduction divisions may be something of an accident when due to chance irregularities of chromosome distribution. On the other hand, sterility based on inability of two sorts of germ plasm to work in harmony, whether in the beginning or at the end of a life history, presents problems that are difficult to vision.

The geneticist postulates, as a form of expression, lethal factors in expressing results of his experimental work when sterility appears, and his formulae are of course justified as steps towards an understanding of finalities. The lethals of the geneticist are placed as genes in the chromo-

somes, and it must be confessed that we cannot show any reasons why they may not be there even though their presence adds mightily to the responsibilities of these heavily worked cell structures. We cannot say that slowness of germination on the part of a pollen grain may not be due to the character of substance or lack of substance in some region of a chromosome even though the pollen tube grows through activity of the ectoplast. On the other hand it may be that some lethals are present outside of nuclear structures. Breeding studies should be able to separate cytoplasmic lethals, if such exist, from those that are associated with chromosomes, since the two would be expected to follow different systems in inheritance.

We have treated sterility as though it depended in the last analysis on factors internal to the organism which may be postulated as genes and which express themselves through inheritance. With respect to sterility in hybrids, its association with germ-plasm organization is generally evident. It is a fair question to ask whether sterility ever has a basis other than that of germ-plasm organization. The temptation is sometimes great to lay the responsibilities of sterility to causes outside the organism and thus to separate a sort of physiological sterility from that which has a genetical relation. There are, for example, forms of sterility brought out by disease or by physiological conditions harmful to the plant. Supposing such sterility to be only partial, as is frequently the case, we should not of course expect the inheritance of this acquired sterility for the same reasons that we do not expect the inheritance of an acquired morphological character. So far as I am aware there are no reasons to regard sterility as other than a characteristic dependent upon germ-plasm organization and dependent in the same sense as are morphological characters.

As stated at the beginning of this paper, hybrid material is generally expected to exhibit in some degree sterility of various sorts. Hybrid material is also generally expected to show its heterozygous nature in breeding by throwing a varied assortment of forms in its progeny. There is coming to be recognized, however, a type of hybrid that reproduces itself perfectly, throwing at most only occasional variants. Such hybrids satisfy fully our concept of a species as a kind of animal or plant which breeds true. They are *impure* species because their germ plasm in the diploid condition carries different sets of genes affecting characters other than those of sex. The *pure* species in contrast has a germ plasm carrying two similar sets of genes each contributed by one of the parents and each with the same genetical constitution except when genes responsible for sex and sex-linked characters are concerned.

Because the impure or hybrid species is of particular interest in relation to the problems of hybrid sterility I must discuss certain phases of this subject although they have been treated with some fullness in my earlier paper "Species, pure and impure." [2]

[2] Davis, B. M. Species, pure and impure. Sci., n. ser. 55: 108–114. 1922.

The impure species, although hybrid in its germinal constitution, breeds true because only such gametes unite and give progeny as will reproduce the heterozygous constitution of the parent plant. Other types of gametes to be expected from the segregation divisions of meiosis either do not develop or fail to function for one of various possible reasons, or, if they do unite, the zygote either can not develop at all or it produces an embryo or seedling which can not mature. In short, there are breakdowns at one or more of various critical points in the life history, and thus some form of sterility eliminates the development of all or nearly all groups of segregates possible to the hybrid in question, and only such combinations of gametes are effective as will give the genotype of the parent.

This concept of the pure-breeding hybrid is not a fancy. We have excellent evidence that impure species are common in the genus Oenothera and that *Oenothera Lamarckiana* is one of them. Certain lines of Drosophila are known to be impure, and we owe to studies of Muller on such material the theory of balanced lethals which offers the best notion of a mechanism in heredity responsible for the generally true breeding of an impure species and for the appearance of occasional variants which some geneticists call mutants but which are really segregates from the heterozygous stock.

The theory of balanced lethals postulates the presence of two different lethals, for example *x* and *y*, the first in one chromosome and the second in the other chromosome of a synaptic pair. The organism is therefore heterozygous for each lethal. The theory also assumes that each lethal is effective only in double dose. The reduction divisions in such material will give two classes of sperms and two classes of eggs, each class distinguished by the presence of one of the two lethals. Thus there will be sperms *x* and *y* and eggs *x* and *y* and the chance mating of these will give zygotes in the following proportions 1*xx* : 2*xy* : 1*yy*. Zygotes *xx* and *yy*, because they have lethals in double dose cannot develop progeny, but the zygotes with the heterozygous combination *xy* will live and reproduce the impure or hybrid parent type. Thus an impure species or race will breed true and maintain a constant state of hybridism unless the relative position of the lethals is changed by a crossover or unless a lethal becomes ineffective through a mutation. A crossover makes possible a class of zygotes free from both lethals, because sperms and eggs would be of the two classes *xy* and *oo* and the zygotes would be in the proportions 1*xxyy* : 2*xy* : 1*oo*. Through the class of segregates free from both lethals recessive characters would appear if genes responsible for their suppression were removed by way of the class homozygous for both lethals. The appearance of such recessives will simulate mutations although in reality they are manifestations of a process of segregation.

There is not time to carry farther a discussion of the bearing of the theory of lethals on the facts of hybrid sterility. It is not probable that conditions in Oenothera and Drosophila, two groups which have received

exceptional attention, are marked exceptions among animals and plants. Intensive studies on other forms are more than likely greatly to extend our recognition of the presence in nature of impure lines and impure species. Hybridization in itself probably invites the development of lethals in proportion as the mixing of diverse germ plasms disturbs delicate and vital adjustments and creates confusion in orderly processes of development.

The subject of lethals and impure species has come to have particular interest for the student of certain groups of plants which are conspicuous components of floras. The systematics of Oenothera has reached a stage so complex that much material can not be identified in the field and species may be determined only when their behavior is studied in the experimental garden. Systematic studies on violets and brambles have employed similar methods of genetical analysis, and many other groups will require the same sort of treatment. Then there are those large assemblages characterized by high degrees of self-sterility, conditions probably not uncommon in the Compositae. Here the progeny is always or usually crossbred as in all unisexual animals and plants. Again, even when self-fertilization is possible, it has been found in some material that inbred lines are not so vigorous as the outbred and thus conditions favor hybridity.

We open a paper with a discussion of pollen- and seed-sterility in hybrids. We are led at the end to touch upon some of the most complex problems of genetics and taxonomy.

University of Michigan

GENETICAL ASPECTS OF SELF- AND CROSS-STERILITY [1]

E. M. East

With the exception of the war period, the genetical and physiological aspects of self-sterility in plants have been under investigation at the Bussey Institution of Harvard University for the last twelve years; and, as often is the case with material worked intensively, the experiments have opened up so many new problems that they will probably be continued for several more years to come. The opportunity afforded by this symposium for bringing together a summary account of that phase of the work having to do with heredity is very welcome, therefore, both because results as yet unpublished have thrown new light on the subject, and because the previous accounts have been too long and detailed for ready reference. The experiments to be reported on here include only those upon the genus Nicotiana which for the last three years have been carried on by Dr. E. S. Anderson, who gives me permission to refer to his unpublished records. Experiments on some other species which I started four years ago are not yet ready for publication; but it may be stated that the data from these later experiments are in no wise contradictory to what I shall have to say.

The self-sterile species used were *Nicotiana alata* Lk. and Otto var. *grandiflora* Comes, *Nicotiana angustifolia* R. and P. var. *crispa* Cav., *Nicotiana commutata* Fisch. and Meyer, *Nicotiana glutinosa* L., and a species believed to be *Nicotiana Forgetiana* (Hort.) Sand. A self-fertile species, *Nicotiana Langsdorffii* L., was also utilized.

Both *N. Forgetiana* and *N. alata* cross easily with *N. Langsdorffii*. The F_1 plants show no evidence of true sterility, *i.e.*, they form normal capsules full of seeds as readily as do either of the parent species. All F_1 plants are self-fertile; and when selfed produce F_2 populations consisting of about 3 self-fertile plants to 1 self-sterile plant. One may assume, therefore, that self-sterility in Nicotiana is due to the presence of the allelomorph of a dominant self-fertility factor, *F*. When a population is homozygous or heterozygous for factor *F*, it is self-fertile; when a population is homozygous for *f*, that is to say when it is *ff*, it is self-sterile. These results corroborate those of Compton for *Reseda odorata*, and have been strengthened by another similar investigation made by Baur on Antirrhinum.

The other three self-sterile Nicotianas used in the work, *N. angustifolia*, *N. commutata*, and *N. glutinosa*, will not cross readily with self-fertile species, hence the genetic relationship could not be determined. But from

[1] Read in the symposium on "Sterility in Plants," at the joint meeting of Section G of the American Association for the Advancement of Science, the Botanical Society of America, and the American Phytopathological Society, at Cambridge, December 27, 1922.

certain evidence gleaned from studying the behavior of the self-sterile plants among themselves, one is led to the belief that either the primary self-sterility factor exists in various allelomorphic forms each having a different efficacy in causing self-sterility or that secondary factors exist which modify this efficacy.

This variation in the efficiency of the self-sterility factor is a peculiar thing. It does not mean that all plants homozygous for *ff* are not self-sterile, for this is not the case. It means only that there is a considerable variation in the ease or difficulty with which environmental changes can produce the slight indication of self-fertility which I have termed pseudo-fertility. Under normal conditions all plants of these species are wholly self-sterile. When grown with ordinary care either in the greenhouse or out of doors they produce not a single seed after selfing, for the first month or so of the flowering season. Later, a few seeds will sometimes be produced after selfing, and this pseudo-fertility appears as a response to changed environmental conditions more readily in some families than others.

There has been some criticism of the use of the term *pseudo-fertility*. The critics would have it that a plant which produces any seed whatsoever after a self-pollination is self-fertile, no matter if it has shown complete self-sterility in 200 or 300 attempts at selfing under what I term normal conditions. The position of these critics is indefensible. It shows an astonishing ignorance of both genetical methods and genetical results. Furthermore, those who take this position are so handicapped by it that they can make no constructive analysis either of their own or other results on self-sterility, although the data yield to a very simple interpretation when it is understood that this pseudo-fertility is a mere environmental fluctuation having nothing to do with heredity. I might say in passing that every somatic character is affected by external conditions. In ordinary cases, such as flower color, one finds that he must make his records before the flower wilts and fades. In less common examples, like the Drosophila mutant having 12 legs, the individuals must be raised under extraordinary conditions in order to bring out the character—in this case extreme cold is necessary. Another example would be hair characters in the human race. Would these critics say that hair color could not be studied because a man becomes bald? Could it be maintained that I did not inherit brown hair, just because I do not have much of it left now, or because of the prospect that I will not have any left a little later? I propose to sit tight on this proposition against all-comers as a matter of honor.

Speaking seriously, pseudo-fertility in the self-sterile plants with which I have been working is a rare occurrence; but it may be caused by any proper combination of circumstances which tends to make the flowers hang to their stems longer and which tends to make the pollen tubes grow more rapidly. Concretely, it is found when the plants are old and the temperature is between 80° and 90° F.; but these conditions produce more marked

effects on some families than on others. Since there is no difficulty in distinguishing true self-fertility from pseudo-fertility, the phenomenon has its advantages. By pollinating young buds of old plants under proper temperature conditions, one can obtain selfed capsules containing between one tenth and one half of the normal complement of seeds, and thus one can deal with populations produced by self-fertilization in self-sterile strains.

The most interesting problem of self-sterility is the behavior of self-sterile plants when crossed among themselves. Darwin supposed each plant to be so specialized in its reproductive organs that, though it could not be fertilized by its own pollen, it could be fertilized by the pollen of any other plant of the same species. Such a particularized specialization he found difficult to explain. Fortunately it is not necessary to explain it, for it is not what actually happens. The self-sterile strains are made up of groups of plants wherein each individual is cross-sterile with all other plants of the same class and cross-fertile with all plants belonging to any other class. In other words, if plant A is sterile with plant B and with plant C, it may be predicted that plant B will be sterile with plant C; and if plant A is fertile with plant X which in turn is sterile with plant Y and plant Z, then it may be predicted that plant A will be fertile with plant Y and plant Z. I shall not discuss the physiological implications of this peculiar phenomenon, but shall describe briefly the facts emerging from the pedigree-culture experiments.

Perhaps the most important genetic question is whether these classes of plants, wherein the individuals are all sterile with each other, are classes which are analogous to the classes of purples, magentas, reds, pinks, and so on, found in the F_2 generation after a white sweet pea is crossed with a purple sweet pea. That is to say, it is desirable to know whether one is dealing with a case of straight inheritance comparable with other cases of inheritance, but where the members of the classes, instead of being distinguishable by visual methods, can be ticketed and grouped only by the criterion of cross-sterility.

To determine this, a population of plants from a cross between *N. Forgetiana* and *N. alata* was tested for cross-sterility. Only 2.4 percent of cross-sterility was found, a percentage so small that it is not difficult to see how easy it was for Darwin to be misled into thinking that every plant within a self-sterile species was cross-fertile with every other plant. Though computation shows that from 20 to 25 intra-sterile, inter-fertile classes would account for such a small percentage of cross-sterility in random crosses, it is obvious that with such a slow-going means of testing the affinities of each individual a clear analysis of a population containing so many groups is impracticable. If the behavior of these self-sterile plants is governed by mendelizing factors, however, then it follows that a series of self-pollinations or of sister-brother matings for several successive genera-

tions will automatically reduce the number of segregating factors and hence the number of intra-sterile, inter-fertile classes. By taking advantage of pseudo-fertility it ought to be possible theoretically to obtain a population of plants belonging to a single class, a population in which every individual is cross-sterile with every other individual, though in this population each individual may fertilize the gametes or be fertilized by the gametes of other populations. These results, predictable on theoretical grounds, were actually obtained. A dozen or so populations with only two intra-sterile classes have been raised and studied, and two populations consisting of a single intra-sterile class have been found. Dr. Anderson has nearly completed an analysis of the precise method by which the controlling factors are inherited. It does not seem advisable for me to discuss these results in detail here as they will be published shortly, but the main facts bearing on the general genetic problem of self-sterility can be stated in a very few words.

First, the behavior of reciprocal crosses is the same. If the pollen of A is sterile on B, then the pollen of B is sterile on A. This is an important fact. In many of the populations studied, segregation and recombination of factors for pistil length was such that extremes of 25 mm. and 70 mm. were found. But under the usual conditions for carrying on the work, conditions under which pseudo-fertility was not a disturbing feature, incompatibility of the short-pistiled plant for pollen from the long-pistiled plant was just as marked as when the reverse cross was made. And, similarly, interclass crosses were just as easy to obtain when the long-pistiled plant was the mother as when the short-pistiled plant was used. Dr. Anderson believes that this result is aided by a high positive correlation between the length of "life" of the flower and the length of its pistil. Be that as it may, the growth rate of the pollen tubes in incompatible matings is so slow that even if the flower from a short-pistiled plant remained on the stem for as long a period as with its long-pistiled sister, the tube does not have the opportunity to traverse more than two-thirds of the pistil distance. It is possible, however, so to control conditions that one may sometimes obtain seed from a particular mating when it is impossible to obtain it after a mating made the reverse way. For example, if plant A, a plant in vigorous condition and at the height of its flowering season, is used as the pollen parent on an incompatible plant B, a plant in weakened condition at the extreme end of its flowering season, some seed will be obtained as a manifestation of pseudo-fertility; but the reciprocal cross is impossible.

A further conclusion may be derived in part from the fact that reciprocal matings are identical when not interfered with by external conditions whose effects are fairly well known and for which reason are controllable and their results predictable. It is to the effect that, though the gametes formed by a particular plant may be packed with many different combinations of genes, as proven by the variable population of zygotes produced, as far as

their activities before fertilization are concerned they behave as if they were all alike. Pollen grains from a single plant may be of variant constitutions with reference to their transmission of qualities to the next generation, therefore, but they show no *selective* fertilization. They are controlled in their gametophytic activities by the genetic constitution of the mother plant on which they are formed. For this reason a genetic experiment conducted in such a manner as to have the critical matings made with pollen from a single plant will give the results to be expected from *chance* matings of germ cells. But one can easily imagine types of genetic matings where the results would be vitiated by not keeping this fact in mind. Suppose, for example, that one desired to make a test mating on plant *A* with pollen from plant *B*. Plant *B* produced little pollen, however, and additional pollen from plant *C* was used because plant *C* was assumed to have the same constitution as plant *B* with regard to the particular factors under investigation. Unfortunately the pollen tubes from the pollen of plant *C* grow faster than the pollen tubes from the pollen of plant *B*, and the resulting zygotes are all sired by plant *C*. Naturally, no geneticist in his right mind would make a test in this way, but nevertheless it may be well to have in mind its possibilities.

Second, the factors which govern the behavior of self-sterile plants are strictly inherited, and are transmitted in accordance with a definite Mendelian mechanism. Class *A*, for example, is class *A* wherever found. A single class has been identified by the cross-sterility test through three successive generations by Dr. Anderson; and the same class has been found in collateral families as far removed as fourth cousins. Thus the behavior of these factors controlling a peculiar physiological difference is exactly the same as that to be found where visually identifiable morphological differences are found.

Third, the genes which govern the behavior of these plants in crosses are numerous. About fifteen classes have been proven to be genetically distinct from each other by proving *each* class to be fertile with *every* other class. Eight or ten other classes have proven to be distinct from all other classes with which crosses have been made; but since every possibility of linking them up with known classes has not been tested, it can not be maintained that they must belong to separate groups. Arguing the matter as a problem in probabilities, however, it can be said that the chances are greatly in favor of there being more than twenty genetically different intra-sterile groups of self-sterile plants in *Nicotiana Forgetiana* and *Nicotiana alata*.

It is greatly to be regretted that it has been impossible to test out thoroughly a good random sample of individuals in these two species. When our work on the self-sterility problem was resumed after the war, only two packets of *N. alata* seed and one packet of *N. Forgetiana* seed would grow. The pedigrees of these seeds were such as to make it highly

probable that each population had been so selected as to reduce the number of intra-sterile groups materially. And so it proved. There were only three or four such classes in each species; and the interesting thing is that no single class of one species was found in the other species or in the classes observed in the descendants of the original hybrids between them which had been made between 1910 and 1912. We can not say therefore whether *N. alata* considered as a species contains any factors governing the behavior of self-sterile plants which are the same as those possessed by *N. Forgetiana*, or not; but from the results obtained we are justified in believing that each species does contain a relatively large number of such factors. They contain such a large number of these factors that the practical result of making random crosses in an unselected population is to obtain such a high percentage of success as to make one believe, with Darwin, that each plant is fertile with the pollen of every other plant.

In conclusion I will say only this: though the study of self-sterility in detail has opened up various fruitful lines of research of which no one can see the end, and though an adequate physiological interpretation of the behavior of incompatible pollen tubes as compared with compatible pollen tubes has not been forthcoming, the genetical problem in its narrow sense, that is to say the problem of the mechanism of its heredity, may be said to be solved. The corroborations of our results coming in from Europe on other species are too exact in detail for one to feel that there is much weight in the criticism that these results refer only to four species of Nicotiana. One can only hope that they will be helpful not only to genetic theory, but to the practical problems of the orchardist who has to deal with self-sterile fruits. They do indeed show why whole varieties of asexually propagated fruits are self-sterile. And, further, the work on the causes and control of pseudo-fertility points the way to a practical method of orchard procedure.

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STERILITY IN RELATION TO HORTICULTURE[1]

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The sterility problem in horticulture will be discussed from the standpoint of those factors which limit the crop. Since most of the principles involved are encountered in pomology, the discussion will be limited to this field. Recent investigations have dealt with many of the causes of what the fruit-grower designates as a crop failure. In the broader sense the sterility problem has its setting in the factors which influence the initiation and formation of fruit buds as well as in those which bear upon the set of fruit. It will be evident that, in all of those fruits in which the edible portion includes a ripened ovary, bloom must precede the production of fruit. Let us for the moment, then, keep in mind the point of view of the horticulturist in analyzing the causes which bear upon the set of fruit. Before taking up the discussion of the individual fruits, emphasis will be placed for the sake of clearness upon some general considerations.

First: The variety is the unit in horticulture. Varieties may be discussed in the classroom and in the laboratory in terms of species, but in the orchard and on the market the variety is the unit. The present tendency, however, in breeding horticultural plants to make so many interspecific crosses will tend to bring the characteristics of the species to the foreground, and at the same time, especially in "wide crosses," to increase the difficulties in the setting of fruit because the hybrid condition may have an important bearing upon the formation of pollen and embryo sac.

Second: The ratio between the number of flowers produced and the number of fruit to set, or to mature, varies in the different fruits. In the apple or plum, for instance, a set of five to ten percent of a full bloom is sufficient for a crop. It would be physically impossible for the tree to mature a fruit for each flower in the apple, pear, plum, or peach. On the other hand it is possible for all the pistils in the grape, raspberry, blackberry, strawberry, or currant to set and mature. Likewise, the number of seeds necessary for fruit development varies. Some fruits are seedless, like the banana and the seedless grape; others, like the peach or plum, require a single seed for development; while in the apple and pear the number of seeds present in ripe fruit differs with the variety and also from season to season. In the strawberry or raspberry, where the development of the edible fruit reaches perfection only when a high percentage of the achenes or drupelets set, many more ovules fail to develop in marketable fruit than

[1] Read in the symposium on "Sterility in Plants," at the joint meeting of Section G of the American Association for the Advancement of Science, the Botanical Society of America, and the American Phytopathological Society, at Cambridge, December 27, 1922.

is generally supposed. It will be seen, therefore, that the opportunity for development of both pistil and seed has an important bearing upon the subject at hand.

Lastly, fruit-setting is complicated by the fact that fruit-bud initiation and development accompany fruit-production in some of the fruits, making it necessary to maintain conditions favorable to both. Where fruit buds are formed before dormancy, winter killing, especially in the peach, plum, and cherry, may exceed all other factors in reducing the crop.

When planted alone and pollinated with its own pollen, a variety is self-fertile or self-sterile. Likewise, in mixed plantings the relationship with other varieties is cross-fertile or cross-sterile. The self- or cross-relationship is definite in some combinations and in others is intermediate or variable. With these relationships in mind, let us note briefly the extent of sterility in each of the different fruits as indicated by the more recent investigations.

Status of Sterility in the Different Fruits. In the apple the status of self- and intervarietal-sterility or fertility is not so well defined as in some of the other fruits. The self-sterile, self-fertile, cross-sterile, and cross-fertile conditions occur in all different degrees. Other factors limiting production are alternate bearing, or the so-called "off year," the coming into bearing late of some varieties, as Northern Spy, and the light bearing, even of older trees, which so often happens with Black Twig. The flower of the apple is perfect, and in most varieties the pollen is abundant. Bagging experiments indicate that about two thirds of the varieties are self-sterile (Lewis and Vincent, 1909; Gowen, 1920; Dorsey, 1921). Cross-sterility has been reported in only a few combinations and does not appear to be as important as self-sterility. When there is a full bloom a ten percent set is sufficient for a crop. Cultural conditions have an important bearing upon the formation of fruit buds as well as upon fruitfulness. In the apple, then, self- and cross-sterility, alternate bearing, the light setting of fruit buds, and coming into bearing late are the important considerations. Any one of these, or two or more, acting together may be limiting factors in production.

The pear has many characteristics in common with the apple. The flower is perfect, and normal pollen and pistils are produced in sufficient quantities. A study of 36 of the principal varieties showed that out of this number 22 were self-sterile (Waite, 1895). As in the apple, varying degrees of self- and cross-sterility exist. The size of fruit is correlated with the number of seeds. Cross-pollination is essential to fruitfulness in a number of the most important varieties. Self-sterility was first demonstrated in the pear by Swayne in 1828.

In the peach, sterility has not been found to be an important commercial problem. Investigations at a number of stations indicate that most varieties are self-fertile and cross-fertile, or at least sufficiently so to give an adequate set for a crop (Whitten, 1913; Fletcher 1909–10; and others). On the other hand, in California all varieties of the almond tested were

found to be self-sterile, at least in certain years (Tufts, 1919). Some of the varieties were also cross-sterile.

The varieties of the quince appear to be both self-fertile and cross-fertile.

The plum presents some unusual features in sterility. With but few, if any, exceptions, self-sterility is absolute so far as tests have been made in the varieties of *Prunus americana*, *P. nigra*, *P. besseyi*, and *P. triflora*. In *P. domestica* only about one half of the commercial varieties set fruit when planted alone (Sutton, 1918). Most varieties are cross-fertile, but some cross-sterile combinations have been demonstrated (Dorsey, 1919 *b*). The flower is perfect and the bloom is profuse. Mixed plantings are necessary to insure a set of fruit with all except those of the European varieties which are self-fertile. As a result of unfavorable weather at bloom, lack of nutrition, and defective pollination, crop failures are frequent.

Judging by the varieties grown at present, sterility seems to be acute in the sweet cherry. All the varieties tested in Oregon were self-sterile (Gardner, 1913), and many of the most important varieties were cross-sterile. Pollen and pistil development appear to be normal. Tests made in other regions show some variation in the degree of self-sterility of the different varieties. Great care must be taken to get the proper varietal relationship in the orchard to avoid light crops from this source.

In the grape the named varieties can be thrown into two distinct groups, one of which is self-fertile and the other self-sterile. The self-sterile and also the cross-sterile condition are associated with the reflexed stamens and defective pollen. The flower type furnishes a reliable guide for mixing varieties in planting to insure pollination. The importance of self-sterility in the grape and the care which must consequently be taken in planting are shown by the fact that out of 132 of the more important commercial varieties 37 have reflexed stamens.

The unfruitfulness of many varieties of the strawberry when planted alone is widely known. Here, as in the grape, self-sterility is correlated with flower type. Where normal pollen is produced there appears to be no incompatibility in either the self- or the cross-relationship (Valleau, 1918). On account of the unfruitfulness of the pistillate varieties, breeders have favored the staminate types; out of 62 of the varieties most extensively grown, only 10 are classified as pistillate (Fletcher, 1917). The dioecious condition in the strawberry also influences the functional activity of anther and pistil. Fertility, either self- or cross-, decreases in the later flowers of an inflorescence to such an extent that only the primary or secondary flowers form fruit. This condition has been best illustrated in unnamed seedlings at the Minnesota station which were of course discarded. Most varieties produce pollen in abundance, and aborted pollen is found to the greatest extent in the staminodia.

Tests made with the brambles show that self-sterility exists to a much greater extent than has been suspected. Even in carefully controlled

crosses the number of achenes which develop is surprisingly limited. In North Carolina, 11 out of 15 varieties of dewberries and 4 out of 16 of the blackberries were found to be self-sterile (Detjen, 1916). In mixed plantings Ancient Britain sometimes sets lightly. In the brambles a considerable number of drupelets can fail to develop before the berries become unmarketable. Counts made with ripe red raspberries (varieties Cuthbert and Latham) showed that as many as ten to fifteen percent of the drupelets had failed to develop in apparently perfect berries; when the percentage ran higher than this, both size and uniformity were affected.

In the currant and the gooseberry, self- and cross-sterility results in the loss of flowers from the clusters, especially at the tip, and in small fruits with few seeds. Imperfect clusters vary with the season and variety, but pollination and position appear to be important factors. In the black currant the pollen is sticky, and artificial pollinations resulted in nearly a perfect set in some varieties (Wellington, Hatton, and Amos, 1921; Hatton and Amos, 1921). In these varieties the length of the style proved to be an important consideration, because self-pollinations took place when the style was long enough to bring the anthers in direct contact with the stigma. Of the black currants studied, all varieties were self- and cross-fertile. The dropping of flowers, generally from the terminal part of the cluster, is one of the most serious difficulties in the currant.

This phase of the subject might be discussed further, but the above summaries will suffice to illustrate the status of fruit-setting in the principal fruits. It will be seen that the sterility problem is different in each and that it varies in degree of importance. A critical survey of the general condition reported above indicates that the factors which condition fruit-setting may be thrown roughly into three main categories, namely, weather, genetics, and nutrition. These will be considered more in detail in the order of mention.

The treatment of these will be clearer if a classification is made at this point of the possible fate of flowers. A study of the life history shows three distinct periods of dehiscence. These are: (*a*) the first drop, which includes all pistils in which growth is stopped before the embryo sac is completely formed; (*b*) the second drop, which includes all pistils in which fertilization has failed to take place; and (*c*) the third, or June, drop, which is made up of those fruits in which the embryo has aborted. This classification does not take into account winter killing or spring frost injury. The effect of the loss of pistils at the different drops will vary with the fruiting habit. It will be seen, then, that there are certain definite causes to which the loss of flowers which do not mature must be attributed.

The Relation of Weather to the Set of Fruit. Unfavorable weather causes more crop failures and irregularities in production than any other single cause. Fruit buds may be injured by low temperatures during dormancy or after growth has started following the break in the rest period, in late

winter or early spring. Freezes and frosts are destructive to flowers and young fruits, but periods of low temperature during bloom may be just as damaging to the crop. Storms of short duration do not necessarily interfere seriously with the set. Strong winds, rains, cloudy weather, and low-temperature periods influence pollen dissemination directly, and indirectly through hindrances to insect activity.

In addition to the factors mentioned above, weather may have a detrimental influence upon the processes taking place at bloom, especially by delaying them. The length of time that pollen is available after dehiscence varies from a few hours to a day or so according to the weather and to the succession in the opening of the anthers. In most seasons there appears to be sufficient dissemination of pollen if it is available.

Stigmas remain receptive under orchard conditions from two to six days. This time may also be considered as the length of life of the stigma. Delays in pollination subtract just so much from the time left before the abscission of the style or disintegration in the egg, which in the apple begins as early as 120 hours after bloom (Knight, 1917), while in the plum the egg appears normal two weeks after the bloom and can still be recognized 33 days afterward (Dorsey, 1919 *b*). The rate of pollen-tube growth becomes of vital importance in relation to fruit-setting when pollination is delayed, when the length of life of the egg is short, or when the growth rate of the pollen tube is retarded by low temperatures during bloom.

On account of the time limit set by the period of activity of the processes taking place at bloom, adverse weather affects fertilization chiefly by causing delay. This appears to be more of a hindrance than direct injury from rain or wind. Aside from killing by frosts and freezes, the effect of weather is indicated at the second or the non-fertilized drop by the loss of flowers in the apple, plum, peach, or cherry, by imperfect clusters in the grape or currant, and by imperfect fruits in the strawberry and raspberry.

Genetic Considerations. The genetic factors inherent in horticultural material which affect the self- and cross-relationship are encountered in dioeciousness, in self- and cross-sterility, and in aborted sex structures. It was shown in the discussion on the status of sterility in the different fruits that these considerations present a serious problem to the grower in a large number of the most important varieties.

The dioecious condition is encountered in the grape and the strawberry. Since incompatibility is practically absent in both, the flower type may be taken as a guide to both the self and intervarietal relationship. Intermediate stages in stamen development which produce defective pollen and apparently normal pistils which, however, do not function (Valleau, 1918) have been confusing to growers. Likewise, in the grape the pollen borne by the reflexed stamens of the pistillate varieties, while forming no germ pore but having a defective generative nucleus (Dorsey, 1914), yet has much the same external appearance as other grape pollen. The failure to recognize

the unfruitfulness of the pistillate varieties has been expensive in many instances, and this mistake is still frequently made in new plantings.

Self- and cross-sterility as found in the apple, cherry, plum, and other fruits is the type resulting from slow pollen-tube growth. It has not been demonstrated as yet whether the gametes would unite if brought together. Incompatibility does not appear to be influenced greatly by nutrition. In such extreme cases as the native plums, self-sterility has not been changed to self-fertility under the conditions of any of the tests, and in controlled crosses self- and cross-sterility are encountered on the same tree with cross-fertile combinations. Pollen germination takes place in both the self-sterile and cross-sterile relationship, although tube growth is slow and variable in length from different grains. The effect of incompatibility on the crop appears at the second drop and may be as detrimental as that of dioeciousness.

Pollen abortion is extensive in the hybrid varieties. Aborted grains become turgid in contact with a receptive stigma but do not send out tubes. Pistil abortion, however, does not appear to be so extensive. In some of the hybrid plums, all the pistils develop normally while more than half of the pollen may be aborted. If the chromosome combinations condition development in the pollen, why not also to the same extent in the egg? The probable explanation for this difference lies in the tetrad of megaspore nuclei where a replacement could take place if any one of the four cells could form the embryo sac in case the others were aborted. This adjustment would theoretically allow for only one fourth as much abortion in the embryo sac as in the pollen grain. It is interesting to note in this connection that deficient nutrition causes more extensive pistil abortion in the plum than pollen abortion. Pollen abortion has but little influence upon fruit-setting, except in those few cases where it is complete, because of the quantity of normal pollen produced by most fruits.

Embryo development may also be conditioned by genetic factors. The June or third drop is composed for the most part of young fruits in which the embryo ceases growth, and consequently is a measure, at least in some instances, of genetic influences although it is also greatly affected by nutrition. To illustrate, in the cross Compass × Yellow Egg 652 flowers out of 1,327 set, but only 8 matured, as contrasted with the cross Compass × Burbank in which 116 fruits set out of 175, and 114 matured. The influence of embryo abortion is much more sharply defined in the fruits with one seed than in those with more than one, and this phase of the varietal relationship must be considered along with many others in finally deciding upon pollenizers.

It will be seen, then, that fruit-setting is influenced by genetic considerations which are independent of the influence of weather or of nutrition. These may affect the first drop through abortion in the embryo sac, the second drop through slow pollen-tube growth as a result of incompatibility,

and the third through embryo abortion. As to just how far genetic factors enter in the failure of so many normal-appearing pollen grains to germinate or of apparently normal seeds to grow, it is difficult to state because so many other considerations also enter.

Nutritional Considerations. The research on the relation between nutrition and sterility, or the broader question of fruit-setting, while not easy to summarize, points to a solution of many phases of the problem. The results include and overlap some things which have heretofore been given other interpretations, and have progressed from a study of the effect of nutrition on the plant as a whole to a study of the effect on the fruiting unit, such as the branch, spur, node, or even the individual flower. Nutritional studies are complicated by the variations encountered in seasons, soils, moisture supply, and in the nutritional requirements of the several horticultural crops.

Some things, however, appear to be well established. The importance of size of plant or tree in relation to yield is becoming recognized, especially in the tree fruits. The primary factors in obtaining size appear to be nitrogen and soil moisture. The whole orchard program should be directed toward a control of these two factors as they bear upon the extension of the fruiting area, fruit-bud initiation and formation, fruit-setting, fruit maturity, dormancy, and hardiness. The early period of rapid growth, of the tree fruits particularly, should be kept in mind, because fruit-bud initiation and formation accompany the period of vegetative extension. While many of the details have not as yet been worked out, it appears that the most favorable conditions for growth, fruit-bud initiation and development, and fruit-setting and maturity may be somewhat conflicting. The conception of the carbohydrate-nitrogen balance (Kraus and Kraybill, 1918) furnishes a workable basis for cultural practices, and the control of fruitfulness depends primarily upon proper pruning, the application of nitrogen, and the conservation of moisture.

While skilled growers have for some time recognized the unfruitfulness of exceptionally vigorous as well as of exceptionally slow-growing plants, the variation in the response of the different fruits complicates the control of fruitfulness. For instance, in the apple young trees, or old trees heavily pruned, are generally found to be unfruitful, and some varieties may be fruitful when interplanted with others which are unfruitful. In fact, York Imperial is sometimes so near the border line between the fruitful and non-fruitful conditions that some limbs on a tree bear fruit while others on the same tree do not. In the peach, on the other hand, yield is closely correlated with vigor (Alderman, 1915). Likewise, the red raspberry responds very readily to nitrogen (Chandler, 1920) and is seldom oversupplied with it, while the black raspberry and blackberry make less response. In general, however, these differences are limited, and up to a certain point in all species, size of fruit and yield accompany strong growth.

One phase of sterility quite disappointing to growers, and due primarily to deficient nutrition, is the production of a heavy bloom from which no fruit is set. This is encountered in the apple, in the plum, and probably to a lesser extent in other fruits, and may include a part of the flowers or all of them. While the effect of deficient nutrition may be different in the several fruits, in the plum the method of expression is pistil abortion (Dorsey, 1919 *b*). The same is also probably true in the apple. In the strawberry and the raspberry, deficient nutrition may result in imperfect berries on account of the poor set of achenes or drupelets. A different condition is encountered in the currant, in some of whose varieties there is as much as ten days to two weeks between the time of opening of the basal and of the terminal flowers. In such instances, the currants at the base enlarge considerably before the terminal flowers open, with the result that the latter drop leaving the bare stem of the cluster. The status of individual flowers in the currant has much in common with the more extreme condition encountered in the apple or plum. In all the fruits which produce an excess of bloom, the position of the individual flower appears to have much to do with its opportunity for development. In the plum, for instance, the flowers borne on the terminal growth come into competition with fruiting laterals, formed soon after bloom at each node, with the result that flowers borne in this position seldom set. Some tests were made at the Minnesota station with the currant to determine the relation between the position of the flower and setting. When the basal flowers were removed from selected clusters before they opened, the terminal ones set, although they had fewer seeds and were smaller. On the same bush under the same field conditions, terminal flowers on clusters not so treated dropped.

With the carbohydrate-nitrogen conception as a background, the trend of the more recent investigations may be illustrated by the work with the apple. Some varieties are known to growers as annual, and others as "off-year" or biennial bearers. For a long time thinning has been advocated as a means of preventing exhaustion and consequently inducing annual bearing. In New York, four years' thinning did not make Baldwin more regular in bearing (Gourley, 1915). Similar results were obtained in West Virginia with Grimes and Delicious (Auchter, 1919). Spurs which fruit one year seldom bloom the next (Auchter, 1919; Roberts, 1920), and recent investigations indicate that, if thinning is to influence fruit-bud formation on spurs which have bloomed, the removal of buds or flowers at least before they set (Roberts, 1920) rather than of the fruit, is required (Crow and Edit, 1921). The suggestions which have come from the later studies as to methods of correcting the "off-year" include good cultural methods (Gourley, 1915), pruning out crowding branches and cutting back to rejuvenate the spurs (Roberts, 1920), stimulation of alternating spur blooming by pruning and fertilization (Roberts, 1920; Crow and Edit, 1921), and obtaining the normal fruit-spur formation on the two-year-old wood

(Roberts, 1920). While tree performance during the bearing and "off-years" indicates marked variability in the physiology of the tree as a whole, spur analyses and defoliation experiments show a similar difference more locally (Heinicke, 1917; Wiggans, 1918; Hooker, 1921, and others).

Control Measures. It may be of interest to investigators in other fields to note briefly some of the methods of controlling sterility which have been worked out to date. The remedy is of course suggested largely by the cause. Means of counteracting the influence of unfavorable weather have been given less attention than nutritional genetic causes. Orchard heating has been used more in the West than in the East and is effective only within a limited range. There may be some advantage in some seasons in extending the period of bloom either by a succession of varieties or by an extended period of bloom with the variety, but this is not always effective in avoiding the effects of prolonged adverse weather. There may be some promise of control within narrow limits from the use of pollenizers whose pollen will develop tubes at lower temperatures than others. This suggestion comes from some of the hardier plums from northern sources. There is a still more readily available remedy in the early application of nitrate of soda, which has given some indication of effectiveness in recent preliminary tests. All told, however, weather will still prevent the set in spite of any of the remedies yet tried when extreme conditions are encountered.

Given favorable weather and growth conditions, a remedy for the genetic causes of sterility is found in mixed planting. This phase of sterility has been given considerable attention, and control measures are well known as a result of the studies made of the self- and cross-relationship of varieties. The effect of aborted pollen, the dioecious condition in the grape and the strawberry, as well as incompatibility, are all counteracted by properly mixing varieties. In fact, the problem can be eliminated in the grape and strawberry by the selection of varieties with perfect flowers. Two methods have come into common use in mixing varieties for pollination purposes—top working and inter-planting. The former is usually adopted in older plantings to correct mistakes and the latter in new plantings. Serious errors have been made in some orchards by planting trees or vines alternately in the rows instead of in narrow blocks or strips. When varieties are planted alternately in this way, both spraying and harvesting are made more difficult. In making mixed plantings careful attention should be given to the relative time of dehiscence of the anthers and to the receptiveness of the stigma.

The details of bringing about the optimum nutritional conditions for fruit-bud initiation and formation, fruit-setting, and fruit-development have as yet only been approached. The control of the carbohydrate-nitrogen-moisture ratio appears to be a workable point of attack. The investigations to date are sufficiently definite to indicate that each of the fruits presents a problem different in many respects. On account of the

bearing on the whole orchard program this field needs further intensive investigation.

An attempt has been made in the preceding discussion to survey the general question of fruit-setting and to evaluate the different factors which have a bearing upon it. It will be seen that these can be assigned to three general causes each of which although influenced by the other may become the dominant consideration. The total effect of these in reducing the crop can best be appreciated by those familiar with reduced yields and crop failures. The investigations in some phases of the problem have gone no farther than to emphasize the need of further work. The fact that fruit for the most part is produced under such variable conditions will of necessity make the set uncertain. There are certain factors, however, some favorable to fruit-setting and some unfavorable, which may be regarded as fixed or constant in their relation to fruit-setting. While the investigations in this field have gone far toward devising means of stabilizing the industry, much more remains to be done.

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GENERA OF NORTH AMERICAN FABACEAE
I. TRIBE GALEGEAE

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The tribe Galegeae has been divided since Bentham's time into seven subtribes. Of these one has already been raised to tribal rank, Psoralieae, which is characterized by the glandular-punctate foliage and by the pods, which are one- or few-seeded, usually indehiscent but rarely breaking open irregularly across the middle, never valvate. Another subtribe should be removed as a tribe Indigofereae, having three characters seldom found elsewhere in Fabaceae and never combined in any of the tribes. These are malpighiaceous hairs (appressed hairs, 2-branched with branches forming a straight line, the hairs therefore apparently attached at their middle), appendaged connectives in the anthers, and lateral spurs on the keel-petals. It may be that Glycyrrhiza and one or two genera should be removed also and form another tribe, on account of the indehiscent (though several-seeded) glandular fruit and confluent anther cells.

After the removal of the two subtribes mentioned, the tribe may be characterized as follows:

Herbs, shrubs, or trees, or woody vines, without glandular dotted foliage (except in Glycyrrhiza). The leaves are odd-pinnate or abruptly pinnate, with or without stipels, very rarely with malpighiaceous hairs. The calyx is campanulate to tubular, 5-toothed or 5-lobed, rarely more or less 2-tipped. The corolla is papilionaceous, with 5 petals, the two keel-petals more or less adnate along the lower margins. The stamens are 10, diadelphous or monadelphous, the connective without appendages. The ovary is usually many-ovuled, more or less curved or bent upwards; the style slender and the stigma small. The fruit is several- or many-seeded, 2-valved, usually dehiscent.

As stated before, Bentham and Hooker acknowledged 7 subtribes. These authors were closely followed by Taubert in Engler and Prantl's Natürlichen Pfanzenfamilien. Taubert's key in translation reads as follows, after the Indigofereae and Psoralieae have been removed:

α. Seeds strophiolate. Flowers in 2's in the leaf-axils or forming a terminal raceme (compare Tephrosia and Fordia). — *c.* Brongniartiinae.

β. Seeds not strophiolate.

I. Inflorescence racemose, terminal, opposite the leaves, or on the branches paniculate, more rarely in the axils of the upper leaves or all peduncles or only the lower paired or clustered in the axils. Stipe inside the staminal sheath sometimes surrounded by a small cup-like disk. — *d.* Tephrosiinae.

II. Inflorescence always axillary. Stipe without disk at the base.
Pod flat, when inflated at least the endocarp flat, 2-valved. *e.* Robiniinae.
Pod inflated or bladdery, rarely flat, then 2-celled lengthwise.
Style bearded above; pod inflated or bladdery, not dehiscent or opening only at the apex, more rarely 2-valved, never with a longitudinal partition. *f.* Coluteinae.
Style naked, more rarely hairy like a brush around the stigma; pod 2-celled or nearly 2-celled lengthwise, seldom 1-celled. *g.* Astragalinae.

From this key it is evident that the subtribes are very artificial and that it has been very hard to find really good distinctive characters. It is also evident that these characters have been drawn without taking into consideration the American genera in full, in which they break down repeatedly.

While the strophiole is very well developed in the Brongniartiinae, in fact better so than in any other group in the Fabaceae, strophioles are present, although small, in several species of Cracca.

The Tephrosieae of Bentham (Tephrosiinae of Taubert) is in itself not a natural division, for Barbiera is not at all closely related to the rest, being distinguished by the long-clawed petals, the presence of 2 bractlets beneath the tubular calyx, and the style bearded along the upper side. In fact, it has no close relative in the whole tribe. The paired bractlets are found in Sesban and related genera and in Diphysa, which are all included in the Robinieae of Bentham (Robiniinae of Taubert).

Neither does Krauhnia seem to be closely related to Cracca or Galega, but apparently forms a natural group with several Asiatic genera, especially Millettia.

The position of the inflorescence, being either terminal or apparently opposite the leaves, a character very important in the minds of both Bentham and Taubert, seems to be of little value, for in many species of Galega and a few of Cracca (see explanation under Cracca) the inflorescence is axillary, just as in the Coluteinae and Astragalinae. The other character, the presence of the disk at the base of the staminal sheath, is also unreliable, for, while it is fairly well developed in Cracca and Krauhnia and their relatives, it is not at all in Barbiera.

The Robinieae of Bentham (Robiniinae of Taubert) is distinguished from the Coluteae and Astragaleae (Coluteinae and Astragalinae of Taubert) by the one-celled, two-valved, flattened, not inflated pod, but in the genus Diphysa, included in the first, the exocarp is strongly inflated, forming two lateral elongate bladders, and in Homalobus and Kentrophyta, segregates of Astragalus, the pod is decidedly compressed and fulfills all the characters ascribed to the Robinieae. In Bentham and Hooker's Genera Plantarum the following remarks are made: "In Astragalus paucis [legumen] planissimum est, sed membranaceum et perfecte septatum." This statement applies to Hamosa Med., a segregate of Astragalus found both in the Old

World and America, in which the pod is flat and 2-celled. In Atelephragma Rydb., another segregate found only in America, the septum is incomplete, while in Homalobus and Kentrophyta, both exclusively American, there is not a vestige of a partition and the fruit is exactly like the fruit of Poitoea, Coursetia, or Corynella, placed in the Robinieae.

Both Bentham and Hooker, and Taubert seem to have ignored or else knew imperfectly these segregates of Astragalus when characterizing their subtribes. In Benthamantha, also placed in the latter subtribe, the pod is also similar but with false transversal partitions. In Sesban and Daubentonia, both referred to Robinieae, the pod is hardly compressed, in the former terete or nearly so, in the latter 4-angled. There is therefore no definite line between these three subtribes as constituted by Bentham and Hooker and by Taubert, nor are the Brongniartiae or the Tephrosieae very distinct.

The classification must therefore be remodeled along other lines. I shall give here a tentative reclassification, which will probably be modified when more study has been made on the Astragalinae.

Seeds strophiolate; embryo with a straight radicle; flowers 1 or 2 in the axils of the leaves or in terminal racemes or panicles; calyx subtended by a pair of deciduous bractlets; pods flat; trees or shrubs. — Subtribe 3. BRONGNIARTIANAE.

Seeds not strophiolate (rarely slightly so in species of Cracca); embryo mostly with an incurved radicle; flowers mostly racemose.

Bractlets 2 under the calyx; pods internally with more or less distinct false cross-partitions.

Calyx tubular, with long lobes; petals long-clawed, their blades cuneate at the base, that of the banner oblong, not spreading; pods flat, many-seeded, dehiscent; shrubs with odd-pinnate leaves. — Subtribe 4. BARBIERIANAE.

Calyx campanulate, with short tooth-like lobes; petals short-clawed, at least the blades of the wings with basal auricles; blade of the banner sub-orbicular or broadly ovate; pods not flattened or if flat 2-seeded (Glottidium).

Exocarp of the pod not inflated; hypanthium obsolete, not differentiated from the calyx; shrubs or trees, with abruptly pinnate leaves. — Subtribe 5. SESBANIANAE.

Exocarp of the pod inflated, forming two elongate bladders, one on each side of the pod; hypanthium well developed and differentiated from the calyx, obconic; shrubs and trees, with odd-pinnate leaves. — Subtribe 6. DIPHYSANAE.

Bractlets under the calyx wanting; pods usually without cross-partitions, except in Benthamantha, Sphinctospermum, and Hebestigma.

Base of the pistil or of its stipe usually surrounded by a more or less developed saucer-shaped disk within the staminal sheath; racemes terminal, or terminal and in the upper axils, or opposite the leaves, or if axillary, the leaflets with parallel oblique lateral veins.

Leaflets with parallel or indistinct lateral veins; pods obliquely striate; leaves without stipels; herbs or low shrubs. — Subtribe 1. Craccanae.

Leaflets netted-veined; pods not obliquely striate; leaves mostly with stipels; trees or (ours) woody vines. — Subtribe 2. Millettianae.

Base of the pistil or its stipe not surrounded by a disk; racemes always axillary; leaflets not with parallel oblique lateral veins.

Pods flat, or if terete with prominent sutures, neither inflated nor with even a vestige of a longitudinal partition.

Flowers in fascicles, on short branches arising in the axils of fallen leaves; leaves abruptly pinnate; banner usually enveloping the other petals. — Subtribe 7. Corynellanae.

Flowers in axillary racemes; leaves odd-pinnate, except in some species of Coursetia and occasionally in Olneya; banner mostly spreading. — Subtribe 8. Robinianae.

Pods more or less inflated or completely or partially 2-celled, by the intrusion of one or both sutures.

Style bearded along the upper margin; pods always 1-celled and inflated. — Subtribe 9. Coluteanae.

Style glabrous, or bearded only around the stigma.

Anther-cells not confluent; pods not prickly; plant not glandular-dotted. — Subtribe 10. Astragalanae.

Anther-cells confluent at the apex; pods in ours prickly; plant glandular-dotted. — Subtribe 11. Glycyrrhizanae.

Subtribe 1. Craccanae

Herbs or shrubs, with alternate odd-pinnate leaves, persistent stipules, but no stipels, the leaflets usually with parallel oblique lateral veins. The flowers are racemose. The calyx is campanulate 5-lobed, but the upper lobes are often more or less united. The corolla is truly papilionaceous, the petals are more or less clawed, the banner is broad and spreading. The stamens are monadelphous or diadelphous, inserted on a more or less developed campanulate disk. The style is glabrous throughout or more or less hairy around the stigma. The legume is elongate, flat, 2-valved, usually obliquely striate. The seeds are reniform or transversely oblong, *i.e.*, their longer axis parallel to the axis of the pod.

The subtribe contains, besides the following three genera, a few confined to the Old World. Cracca is cosmopolitan of warmer regions, Peteria is endemic American, and Galega is Eurasian, introduced in the New World.

Stipules not spinescent; lateral veins of the leaflets prominent.
Upper filaments wholly united with the staminal sheath, forming a closed tube; banner in ours glabrous. 1. GALEGA.
Upper filament free, at least at the base; banner strigose on the back. 2. CRACCA.
Stipules spinescent; upper filament free; lateral veins of the leaflets obsolete. 3. PETERIA.

1. **Galega** [Tourn.] L. Sp. Pl. 714. 1753

Perennial herbs. The leaves are odd-pinnate, with semi-sagittate stipules. The flowers are in axillary or terminal racemes with narrow bracts and no bracteoles. The calyx has 5 subequal lobes. The corolla is white or light blue; the banner is obovate-oblong, narrowed below into a very short claw; the wings have an oblong blade with a prominent basal auricle, and a longer claw, and are adherent to the keel at the middle; the keel-petals are obtuse, more or less arcuate, longer than the wings, and united nearly their whole length. The filaments are monodelphous, *i.e.*, all united into a sheath. The ovary is sessile, many-ovuled; the style glabrous; the stigma small, terminal. The pod is linear, terete, 2-valved, sometimes constricted between the seeds. Seeds are transversely oblong, without strophiole.

ILLUSTRATION: Plate XXXIII *A*. *Galega officinalis* L., × 2/3; 1. calyx, 2. banner, 3. wing, 4. keel-petal, 5. staminal sheath, 6. pistil, × 2; 7. pod, × 1; 8. cross section of pod, 9. seed, × 2.

In the Species Plantarum, the genus Galega contained only one species, *Galega officinalis* L., which therefore is the type.

Synonyms:

Callotropis G. Don. Gen. Syst. **2**: 228. 1832. Type: *C. tricolor* (Hook.) G. Don., based on *Galega tricolor* Hook., which is supposed to be the same as *G. officinalis* L.

Accorombona Endl. Gen. 1427. 1841. This was a substitute for Callotropis G. Don., not Calotropis R. Br. 1809. Hence the same type.

The genus consists of 4 or 5 species native of southern Europe and the Orient. Of these, *G. officinalis* is sometimes cultivated as a forage plant and in olden times was used in medicine. It has been found occasionally in the western states from Kansas to Utah, as an escape from cultivation or introduced incidentally among seeds. The genus is closely related to Cracca, differing mainly in the monadelphous stamens. The racemes are mostly axillary, and therefore the genus is, according to Bentham and Hooker, anomalous in their subtribe Tephrosieae, but, as will be shown, this abnormality is found even in species of Cracca.

2. **Cracca** L. Sp. Pl. 752. 1753

Herbs, often woody below, or shrubs. The leaves are odd-pinnate, the leaflets striate, with veins oblique to the midrib and parallel; the stipules

are setaceous, or broader and striate. The flowers are racemose, the racemes are either terminal, with or without smaller racemes in the upper axils, or apparently opposite the leaves, or rarely axillary. The individual flowers are usually in clusters of 2–6 at each node of the peduncle. The calyx is campanulate, furnished with a more or less developed disk; the lobes are five, either subequal or the lowest one longer, the upper two usually more or less united. The petals are clawed; the blade of the banner is suborbicular, more or less sericeous without; the blades of the wings are oblong or obliquely obovate, slightly coherent with the keel, with a more or less developed basal auricle; the keel-petals are more strongly lunate with a larger basal auricle. The stamens are usually partly monadelphous, the uppermost filament at first free from the staminal sheath at the base, adnate to it at the middle, and often separating from it later. The ovary is sessile, many-ovuled, the styles inflexed or incurved, somewhat horny at the base, most commonly glabrous, the stigma terminal. The pod is sessile, flat, 2-valved, many-seeded.

ILLUSTRATION: Plate XXXIII *B*. *Cracca virginiana* L., × 2/3; 1. calyx, 2. staminal sheath, 3. pistil, 4. banner, 5. wing, 6. keel-petal, 7. pod, × 1; 8. pod in cross-section, 9. seed, × 2.

The genus was first established in Linnaeus' Flora Zeylanica 1747. The first species described both in this book and in Species Plantarum is *C. villosa*, which must be regarded as the type.

Synonyms:

Brissonia Neck. Elem. 3: 36. 1790. No type was given, but the genus was based on "some species of Galega L." [Necker's reference indicated the 14th edition of Linnaeus' Systema Vegetabilium.] The first botanist to assign species in the genus was Desvaux (Jour. Bot. 3: 78. 1814), who proposed *B. trapesicarpa*, *B. stipularis*, and *B. coronillaefolia*, but none of these are found in Linnaeus' work named above. De Candolle (Prod. 2: 249. 1825) adopted Brissonia as a section under Tephrosia. Of this section apparently *T. toxicaria* Pers. should be regarded as the type.

Reineria Moench, Meth. Suppl. 44. 1802. Type: *R. reflexa*.

Thephrosia Pers. Syn. Pl. 2: 328. 1807. It was based on 39 species, without definite type. The first species is *T. filifolia*, but the type ought to be sought in the second and larger division with pinnate leaves.

Kiesera Reinw. Syll. Pl. Nov. 2: 11. 1828. Type: *K. sericea* Reinw., which is supposed to be the same as *Tephrosia candida* DC.

Apodynomene E. Meyer, Conn. Pl. Afr. Aust. 111. 1835. Type: *E. grandiflora* (Pers.) E. Meyer, based on *Tephrosia grandiflora* Pers. This is the first and best known species.

Catacline Edgew. Jour. As. Soc. Beng. 16: 1214. 1847. Type: *C. sericea* Edgew.

Macronyx Dalz. Hook. Kew Jour. 2: 35. 1850. Type: *M. strigosus*, which is close to if not identical with *Tephrosia tenuis* Wall. Perhaps this should be excluded from the synonyms of Cracca.

Balboa Liebm. Vidensk. Meddel. 1853: 106. 1854. Type: *Balboa diversifolia* Liebm., the only species.

Seemannantha Alef. Bonplandia 10: 264. 1862. This was a substitute for Macronyx, and hence based on the same type.

Cracca is a large genus, probably containing about 150 species, found in warmer regions of both hemispheres. In North America (including Central America and the West Indies), there are 72 species, of which 65 are native and 7 introduced.

Taubert divided the genus into 4 sections: Brissonia, Reineria, Pogonostigma, and Requienia. Of these the last two, which are not represented in America, should be removed as distinct genera, having 1-seeded fruits.

The principal character by which Taubert distinguished the first two sections was the relative width and length of the calyx lobes. Some species, as for instance *C. leucantha*, which has long and narrow calyx-lobes, really belong to Brissonia instead of Reineria. A better distinction is the inflorescence, which is monopodial in the former and sympodial in the latter. In the monopodial inflorescence the terminal raceme is best developed, but many of the species bear also secondary racemes in the upper axils. In the sympodial inflorescence the terminal raceme develops first, but in the uppermost leaf-axils a branch is produced which in its turn develops a terminal raceme; this is repeated several times, and the racemes therefore appear to be opposite the leaves. In a few species, as for instance *C. rhodantha*, *C. foliosa*, *C. vicioides*, and *C. Brandegei*, the racemes are mostly at the nodes, but neither opposite the leaves nor strictly axillary; they are inserted in the axils but obliquely, *i.e.*, not in the plane determined by the stem and the rachis of the leaves. The monopodial or sympodial character is not perfectly clear.

Several of the species are used as a fish-poison by the natives of the region where they grow, others for poisoning arrows in Southern Africa; still others furnish a blue dye, somewhat resembling indigo.

3. **Peteria** A. Gray. Pl. Wright 1: 50. 1852

Perennial herbs, somewhat woody at the base. The leaves are odd-pinnate with spiny stipules. The flowers are usually in terminal racemes. The calyx is cylindro-campanulate, gibbous at the base above; 5-lobed with the upper two lobes united high up. The corolla is ochroleucous or nearly white, the banner long-clawed with an oblong-obovate blade; the wings have an obliquely oblong blade, slightly auricled at the base, and a slender claw; the blades of the keel-petals are obliquely obovate, obtuse, with a broad, rounded basal auricle. The stamens are diadelphous, with the upper filament wholly free. The ovary is short-stipitate or sessile, many-ovuled, the style with a horny base, inflexed, glabrous, except at the apex, where there is a ring of hairs. The pod is linear, straight, compressed, 2-valved, many-seeded, with thick sutures.

The genus was based on a single species, *P. scoparia* A. Gray, and con-

sists of three species of southwestern United States and northern and central Mexico. In habit the species resemble some species of Astragalus and its segregates, but the racemes are not axillary but terminal, or when an axillary branch is developed become apparently opposite the leaves as in Cracca. The spinescent stipules constitute also a distinctive character. The genus is evidently related to Cracca, but the hair-tufts around the stigma and the less distinct veining of the leaflets obscure the relationship.

ILLUSTRATION: Plate XXXIII *C. Peteria scoparia* A. Gray, × 2/3; 1. calyx, × 2; 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal, × 1; 7. pod of *P. glandulosa*, × 2/3; 8. cross section of the same, × 2; 9. seed, × 2.

SUBTRIBE 2. MILLETTIANAE

Trees or woody vines with alternate odd-pinnate leaves and persistent stipules, usually also with stipels. The calyx is campanulate, 5-toothed, but the lobes are often minute, or the upper 2 and the lower 3 more or less united, forming an upper and a lower lip. The corolla has short-clawed petals, the banner being broad, spreading or reflexed. The stamens are monadelphous or diadelphous, the upper filament free at least at the base. The pod is flat, 2-valved, elongate, several-seeded. Seeds mostly reniform.

Besides the following genus which is represented by native species in the eastern United States and eastern Asia, the subtribe consists of four or five Asiatic and perhaps two African genera.

4. **Kraunhia** Raf. Med. Rep. N. Y. II, 5: 352. 1808

High-climbing woody vines with odd-pinnate leaves, small stipules and stipels. The flowers are in terminal racemes, with deciduous bracts. The calyx is more or less 2-lipped, the upper lip with 2 broad teeth united to near the apex, the lower with 3 longer teeth. The corolla is blue or purple, rarely white, the petals are subequal in length; the banner has a suborbicular blade, reflexed, and with 2 callosities or appendages, the claw is short; the blades of the wings are obliquely obovate, falcate with a large basal auricle on the upper edge and often a smaller one on the lower; the keel-petals are clawed, united at the apex, the blade is lunate with a sharp basal auricle. The stamens are diadelphous, the upper stamen is free or slightly adherent at the middle. The ovary is stipitate, many-ovuled, glabrous; the style inflexed, glabrous; the stigma small. The pod is elongate, flat, 2-valved. The seeds are reniform, without strophiole.

ILLUSTRATION: Plate XXXIII *D. Kraunhia frutescens* (L.) Greene, × 2/3; 1. calyx, 2. stamens, 3. pistil, 4. banner, 5. wings, 6. keel-petals, × 1; 7. fruit, 8. the same in cross section, × 2/3.

The genus was established on *Glycine frutescens* L. without a diagnosis. Synonyms:

Diplonyx Raf. Fl. Ludov. 101. 1817. Type: *D. elegans* Raf., which is regarded as the same as *Kraunhia frutescens* (L.) Greene.

Thysanthus Ell. Jour. Acad. Phila. 1: 371. 1818. Type: *T. frutescens* (L.) Ell., based on *Glycine frutescens* L.

Wisteria Nutt. Gen. 2: 115. 1818. Type: *W. speciosa* Nutt., based on *Glycine frutescens* L. Sprengel (Syst. 3: 255. 1826) corrected the spelling of the name to Wistaria, as the genus had been dedicated to Dr. Wistar.

The genus consists of 5 or 6 species, of which two are native of eastern and southern United States. It is evidently very closely related to the large Asiatic genus Millettia, the species of which are mostly trees or shrubs; only a few of them are climbing.

Subtribe 3. Brongniartianae

Trees or shrubs, with alternate odd-pinnate leaves, stipules, and sometimes stipels. The flowers are axillary or in terminal racemes or panicles. The calyx is more or less 2-lipped, the tube short, the upper 2 lobes united high up, the lower 3 lobes also somewhat united. The corolla has a broad banner. The stamens are monadelphous or diadelphous. The pod is usually elongate, flat, 2-valved, several-seeded. The seeds are erect, *i.e.*, the longer axis of the seed is at right angles to the axis of the pod, with a well developed strophiole.

The subtribe consists of the following two genera and two from Australia. It is distinguished by the well developed strophiole. Its relationship is probably with the Robinianae.

Calyx 5-lobed, the upper 2 lobes united two thirds their length, the lower 3 usually free to near the base; stamens diadelphous. 5. Brongniartia.

Calyx 2-lipped, the upper 2 and the lower 3 lobes united to the apex; stamens monadelphous. 6. Harpalyce.

5. **Brongniartia** H.B.K. Nov. Gen. & Sp. 6: 465. 1823

Shrubs or trees, with odd-pinnate leaves. Stipules are present, but often caducous, the leaflets entire-margined, sometimes with minute stipels. The flowers are normally axillary in small 1- –7-flowered clusters. In some species, however, the upper floral leaves are reduced to the two stipules, which resemble a pair of bracts, and the inflorescence becomes falsely racemose. The individual flowers are subtended by a pair of bractlets, sometimes foliaceous, sometimes reduced to a pair of hair-tufts. The upper two calyx-lobes are united high up, the lower only slightly at the base. The corolla is red, brown, or purple; the banner is broad, its blade orbicular or broadly obovate, short-clawed; the wings are obliquely oblanceolate or obovate, more or less falcate, with a short fleshy claw and a rounded auricle; the blades of the keel-petals are broadly lunate, with a fleshy claw, united from the middle to the tip. The stamens are diadelphous, the alternate ones shorter; the ovary is short-stipitate, the style incurved, glabrous, the stigma minute. The pod is short-stipitate, flat, elongate, 2-valved, usually several-seeded, slightly wing-margined on the upper suture, the valves leathery.

Illustration: Plate XXXIV *E*. *Brongniartia Benthamiana* Hemsl., × 2/3; 1. calyx, 2. staminal sheath, 3. pistil, 4. banner, 5. wing, 6. keel-petals, × 1; 7. pod, × 2/3; 8. seed of *B. sericea* Schlecht., × 1.

The genus was established on two species, *B. mollis* and *B. podalyrioides*, of which the first may be regarded as the type.

Synonyms:

Peraltea H.B.K. Nov. Gen. & Sp. **6**: 469. 1823. Type: *P. lupinoides* H.B.K., now known as *Brongniartia lupinoides*.

Megastegia G. Don, Gen. Syst. **2**: 468. 1832. Type: *M. speciosa* G. Don, regarded as the same as *B. thermoides*.

The genus consists of 37 species, natives of Mexico and Central America, and a few species from South America. One species, *B. oligosperma* Baill., is somewhat abnormal in the genus on account of its hairy few-seeded pods.

6. **Harpalyce** DC. Mem. Leg. 496. 1825

Trees or shrubs, with alternate odd-pinnate leaves and small stipules. The leaflets are entire-margined, petioluled, sprinkled beneath with yellow or orange glands or gland-like scales. The flowers are racemes. The calyx is 2-lipped, with the upper 2 and the lower 3 lobes united to the tip. The banner is rounded or obovate, short-clawed, the wings are very irregular, strongly curved, obtuse, the keel-petals more or less falcate, united to above the middle but the tips free and obtuse. The stamens are monadelphous, but the sheath is split to the base; the anthers are alternately longer and shorter. The ovary is sessile, the style arcuate, glabrous, the stigma minute, terminal. The pod is 2-valved, several-seeded, leathery or woody.

Illustration: Plate XXXIV *F. Harpalyce Loeseneriana* Taub., × 2/3; 1. calyx, 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal, × 1; 7. pod, 8. cross section of the same, × 1; 9. banner of *H. cubensis* Griseb., 10. wing, 11. keel-petal, 12. seed, × 2/3.

The genus was based on an unpublished illustration in Mocino and Sesse's Flore de Mexique of *Astragalus carnosus*. In De Candolle's Prodromus the type is given as *H. formosa* DC., based on the same.

The genus consists of 14 species, *viz.*, 7 from Mexico, 1 from Guatemala, 3 from Cuba, and 3 from Brazil. One species, *H. mexicana*, is abnormal in that the valves of the pod are woody. As the flowers of this species are unknown, it may belong to some other genus. The rest can be divided into three natural groups: (1) Cuban species, in which the petals are fleshy, the keel much longer than the other petals, and the pod is small and narrow; (2) Mexican species (including the one from Guatemala), in which the petals are membranous, the keel is scarcely longer than the banner, and the pod large and broad; (3) Brazilian species, similar to the Mexican but the pod narrower and more or less divided internally by false cross-partitions of spongiose tissue.

Subtribe 4. Barbierianae.

Shrubs, with alternate odd-pinnate leaves, narrow stipules and stipels. The flowers are in axillary and terminal racemes, each subtended by a pair of bractlets. The calyx is cylindric, 5-lobed, the lobes subequal. The corolla is not truly papilionaceous, the petals with long slender claws; the blade of the banner is oblanceolate, not spreading; the wings have elliptic blades, the keel-petals oblanceolate or oblong, obtuse blades, scarcely

falcate, and united above the middle. The stamens are diadelphous, the sheath is straight; the ovary is sessile, the style nearly straight to near the apex, hairy along the upper side above; stigma minute. The pod is flat, straight, 2-valved, transversely septate within.

The subtribe consists of a single genus and a single species.

7. **Barbieria** DC. Mem. Leg. 241. 1825

The generic characters are included in the subtribal description.

ILLUSTRATION: Plate XXXIV *G. Barbieria pinnata* (Pers.) Baill., × 2/3; 1. calyx, 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal, 7. pod, × 1; 8. cross section of the same; 9. seed, × 2.

The genus was based on *Clitoria polyphylla* Poir. or *Galactia pinnata* Pers. Its distribution extends from Porto Rico to Cuba, southern Mexico, Bolivia, and Brazil.

SUBTRIBE 5. SESBANIANAE

Trees, shrubs, or herbs with abruptly pinnate leaves and caducous stipules but no stipels. The flowers are borne in axillary racemes. The calyx is round-campanulate, fully as broad as high, with very short teeth. The banner has a broad reflexed blade and a short claw; the keel-petals are strongly arcuate, united at the middle, but the claws and tips are free. The stamens are diadelphous; the staminal sheath is dilated below and the upper filament knee-bent near the base. The pod is 2-valved but often indehiscent, stipitate and beaked, compressed, usually with cross-partitions between the seeds, but not disarticulate.

On account of the abruptly pinnate leaves this subtribe, as well as the Corynellanae, is somewhat abnormal in the tribe but could not be transferred to the tribe Vicieae, to which it does not show any relationship in other ways. The structure of the flower is practically the same as in many genera of the subtribe Robinianae; the pod, however, is, in most of the genera, but slightly compressed and internally divided by false transverse partitions.

The subtribe consists of the five following genera, which are thus distinguished.

Pods flattened, neither 4-winged nor 4-angled.
- Pods many-seeded, linear, with thick margins; calyx not conspicuously oblique.
  - Pod not torulose.
    - Flowers middle-sized or small; banner suborbicular; blades of the broad keel-petals with an auricle; seeds subcylindric-oblong. — 8. SESBAN.
    - Flowers large; banner elliptic; blades of the rather narrow keel-petals without a basal auricle; seeds reniform-oblong. — 9. AGATI.
  - Pod torulose; banner suborbicular, equaling the other petals; blades of the broad keel-petals without a basal auricle; seeds reniform-oblong. — 10. DAUBENTONIOPSIS.

Pods 2-seeded, with thin margins, lance-elliptic; seeds oblong-reniform enclosed in the inner membranous layer of the valves; calyx decidedly oblique. 12. GLOTTIDIUM.
Pod 4-angled, often 4-winged, not torulose. 11. DAUBENTONIA.

8. **Sesban** Adans. Fam. 2: 327. 1763

Herbs or shrubs with abruptly pinnate leaves and numerous leaflets. The flowers are borne in axillary racemes, with deciduous bracts, and a pair of deciduous bractlets under the flowers. The corolla is yellow or the banner dotted, streaked, or tinged with purple. The banner has often one or two callosities at the base of the suborbicular reflexed blade; the wings are short-clawed, the blades obliquely oblanceolate or oblong, with a basal auricle; the keel-petals have long claws, the blades are strongly and broadly lunate with a lateral auricle on the upper side. The pod is slender, terete or slightly compressed, short-stipitate, many-seeded, with cross-partitions between the seeds, 2-valved. Seeds cylindro-oblong, subtruncate at the ends.

ILLUSTRATION: Plate XXXIV *H. Sesban Sesban* (L.) Rydb., × 1/2; 1. calyx, 2. stamens, 3. pistil, × 2; 4. banner, 5. wing, 6. keel-petal, × 1; 7. pod, × 1/2; 8. cross section of pod; 9. seed, × 2.

The genus was established on *Aeschynomene Sesban* L. Scopoli (Introd. 308. 1777) changed the name to Sesbania, the latter being better Latin form.

Synonyms:

Darwinia Raf. Fl. Ludov. 106. 1817. Type: *D. exaltata* Raf., which has been known under the name *Sesbania macrocarpa* Muhl.

Monaplectra Raf. Fl. Ludov. 106. 1817. This was proposed instead of *Darwinia* in case the latter happened to be preoccupied.

The genus consists of perhaps 20 species of the warmer regions of both hemispheres. It is represented in North America by 4 native and 3 introduced species.

9. **Agati** Adans. Fam. 2: 326. 1763

Small trees, having abruptly pinnate leaves, with many entire leaflets and deciduous stipules. The flowers are large, borne in small axillary racemes, and with two deciduous small bractlets subtending the calyx. The calyx is in structure the same as in Sesban. The petals are comparatively narrower, the banner is without callosities, its blade oval in outline, and retuse at the apex; the blades of the wing- and keel-petals are obliquely lanceolate-lunate, tapering at the base without any distinct auricle. The staminal sheath and the pistil resemble also those of Sesban, except that they are more gradually arcuate. The pod is the same as in that genus.

ILLUSTRATION: Plate XXXV *I. Agati grandiflora* (L.) Desv., × 1/2; 1. stamens, 2. pistil, 3. banner, 4. wing, 5. keel-petal, × 1/2; 6. pod, × 1/4; 7. cross section of the same, × 1; 8. seed in position in the pod, × 1.

The genus was based on *Robinia grandiflora* L.

Synonym:

Resupinaria Raf. Sylva Tell. 115. 1838. Type: *R. grandiflora* Raf. or *Robinia grandiflora* L.

The genus is represented by *A. grandiflora* (L.) Desv. and one or two species closely related to it and perhaps not specifically distinct from it, natives of southern Asia and northern Australia, and by *A. tomentosa* (H. & A.) Nutt. from the Hawaiian Islands.

10. **Daubentoniopsis** Rydb., gen. nov.

Shrubs, having abruptly pinnate leaves, many entire caducous stipules, caducous bracts and bractlets. The flowers are in axillary racemes. The calyx is rounded campanulate, broader than high, its lobes very short. The corolla is yellow, the banner suborbicular, retuse, reflexed, with a short claw, without callosities; the wings are short-clawed, with obliquely oblong blades, without a distinct basal auricle; the keel-petals are also clawed with a lunate, nearly semicircular blade. The stamens are diadelphous, the staminal sheath dilated at the base. The ovary is stipitate, glabrous, the style arcuate, glabrous; the stigma minute. The pod is coriaceous, stipitate, somewhat compressed, linear, several-seeded, decidedly constricted between the seeds and with spongy transverse partitions. The seeds are oblong-reniform, about twice as long as high.

ILLUSTRATION: Plate XXXV *J. Daubentoniopsis longifolia* (Cav.) Rydb., × 1/2; 1. calyx, × 2; 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal, × 1; 7. pod, 8. cross section of the same, × 2/3.

The genus is based on *Aeschynomene longifolia* Cav. Ic. 4: 8. 1797. It is intermediate between Sesban and Daubentonia, having exactly the flowers and seeds of the latter, but the pod is neither 4-angled nor winged. It is constricted around the seeds, but the exocarp is spongy. In Sesban the pod is hardly constricted, the calyx-teeth are more evident, the banner usually has a callosity, and the seeds are different.

The type of the genus, **D. longifolia** (Cav.) Rydb., has a rather peculiar history. It was first described by Cavanilles (*loc. cit.*), and independently by Ortega[1] under the same name. Cavanilles' species was transferred to Piscidia by Willdenow.[2] De Candolle,[3] when he established the genus Daubentonia, included it in that genus, but from the short description given it is evident that he had in mind a yellow-flowered Daubentonia of the southern United States and northern Mexico. In his Prodromus[4] he repeated his error, and besides described on page 265 a *Sesbania longifolia* based on *Aeschynomene longifolia* Ortega. His description fits Cavanilles' plant. When Watson[5] merged Daubentonia into Sesbania, he, influenced by De Candolle's misconception, thought himself forced to give the yellow-flowered Daubentonia a new name, *S. Cavanillesii* S. Wats., as there was already a *S. longifolia* (Ort.) DC. Unfortunately, however, he based this name on *Aeschynomene longifolia* Cav., and technically he gave a new name

[1] Ort. Dec. 70. 1797–1800.
[2] Sp. Pl. 3: 920. 1803.
[3] Mem. Leg. 286. 1823.
[4] Prodr. 2: 267. 1825.
[5] Bibl. Ind. 258. 1878.

to the species of Daubentoniopsis instead. Pollard,[6] following De Candolle's and Watson's interpretation of Cavanilles' plant, was of the opinion that this, being the first one described, should retain the specific name *longifolia*, and therefore proposed the name *S. mexicana* Poll. for Ortega's plant. As the two are the same, he merely added a new synonym to our species of Daubentoniopsis. The yellow-flowered Daubentonia is still nameless.

11. **Daubentonia** DC. Mem. Leg. 285. 1823

Shrubs or trees, having abruptly pinnate leaves, with many leaflets and deciduous stipules. Flower in axillary racemes, resembling closely those of Sesban, but the calyx-lobes are still smaller, the calyx-tube being merely undulate on the margins, slightly ciliate at the teeth. The banner is without callosities, and the wings and keel-petals are without basal auricles. The pod is more or less 4-angled, but somewhat compressed. The endocarp is membranous and constricted around the seeds, the exocarp more or less spongy, the sutures are thick, each produced into two sharp ridges or wings. The seeds are reniform.

ILLUSTRATION: Plate XXXV *K*. *Daubentonia punicea* (Cav.) DC., × 1/2; 1. calyx, × 2; 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal, 7. pod, 8. cross section of the same, × 1.

The type of the genus is *D. punicea* (Cav.) DC., based on *Piscidia punicea* Cav.

The genus consists of about half a dozen species, of which 3 are natives of South America, one of the southern United States and northern Mexico, **Daubentonia Drummondii** Rydb. (*Daubentonia longifolia?* T. & G. Fl. N. Am. 1: 293. 1838), and 1 or 2 of Mexico. I have based it on *D. longifolia?* T. & G., rather than on *D. longifolia* DC., in part as to description, for that name really belongs to *Daubentoniopsis longifolia*, as stated before. One of the South American species, *D. punicea*, has also been found introduced in Florida and Mississippi.

12. **Glottidium** Desv. Jour. Bot. 1: 119. 1813

Annual herbs, having abruptly pinnate leaves, with many leaflets and deciduous stipules. The flowers are in axillary racemes or panicles. The calyx and corolla are almost exactly like those of Sesban, the banner with callosities, the wings and keel-petals of the same shape as those of that genus. The pod, however, is different, broad, stipitate, compressed, 2-valved, and 2-seeded, the valves at last separating in two layers, the endocarp very thin and papery, the exocarp firmer, somewhat inflated, but not bladdery as in Diphysa.

ILLUSTRATION: Plate XXXV *L*. *Glottidium vesicarium* (Jacq.) Harper, × 2/3; 1. calyx, 2. stamens, 3. pistil, 4. banner, 5. wing, 6. keel-petal, × 2; 7. pod, × 2/3; 8. pod in cross section, × 1; 9. seed, × 2/3.

The genus is monotypic and was based on *Aeschynomene platycarpa* Michx., which is the same as *Glottidium vesicarium* (Jacq.) Harper.

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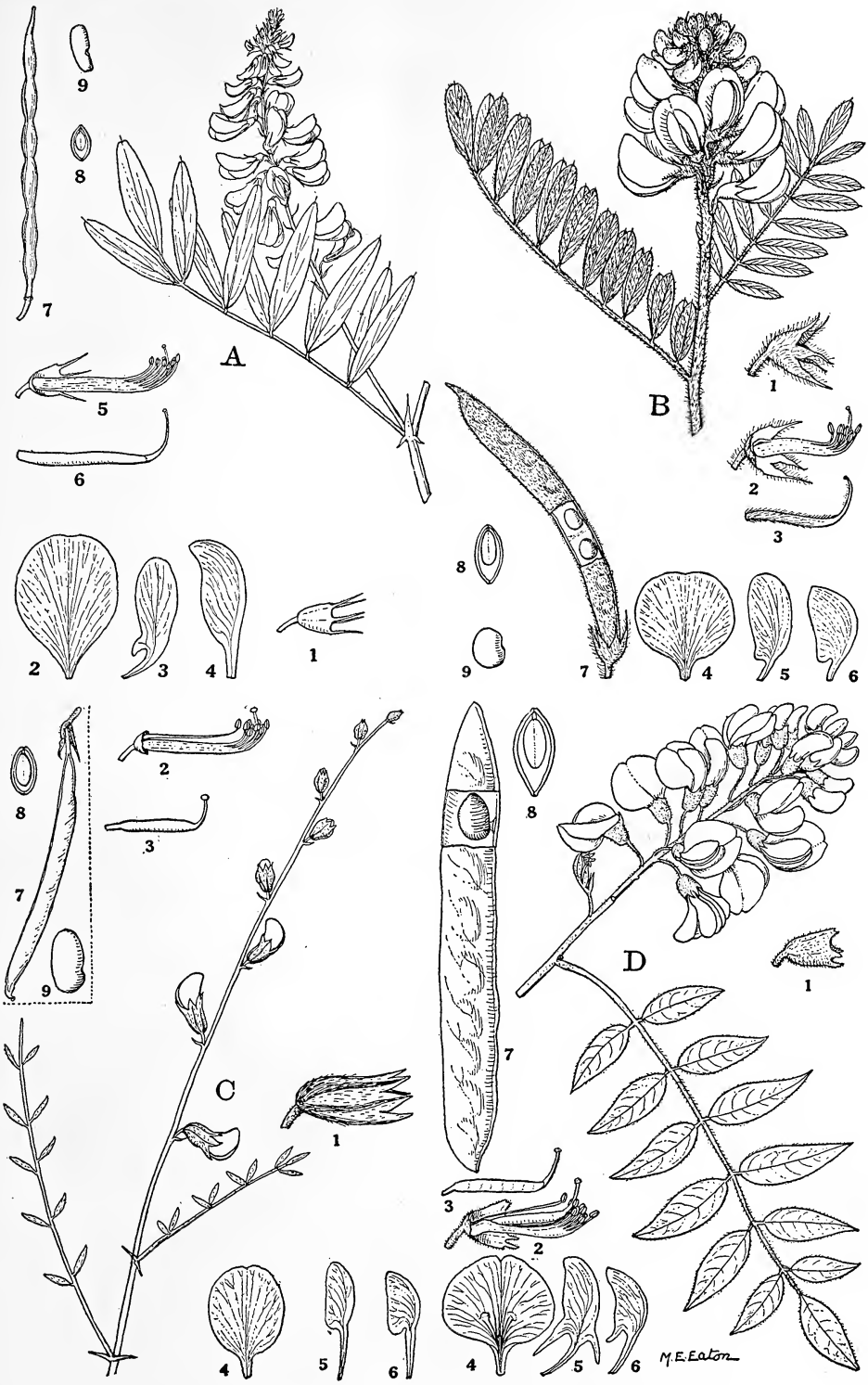
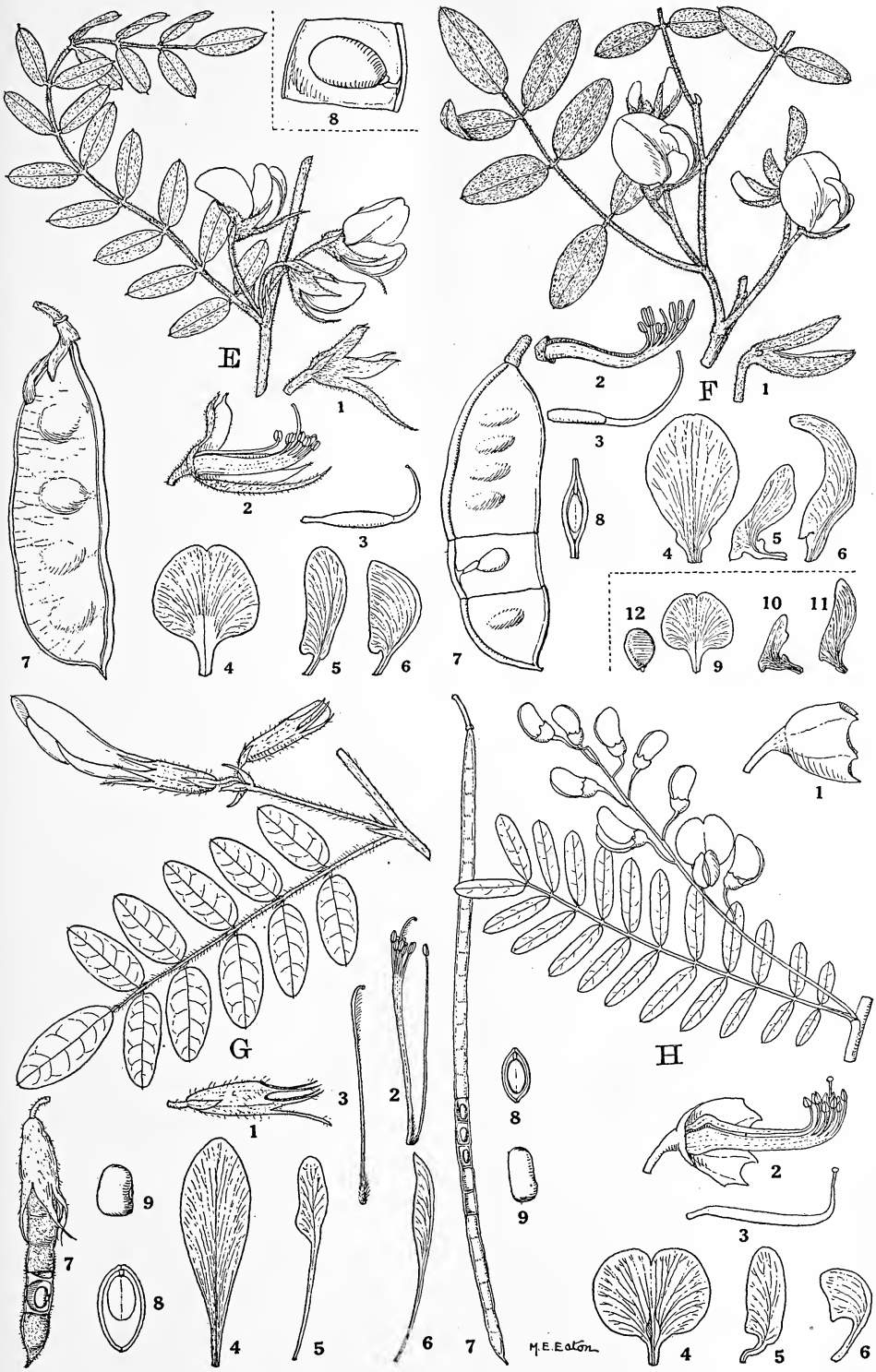
[6] Bull. Torrey Bot. Club 24: 154. 1897.



RYDBERG: NORTH AMERICAN FABACEAE



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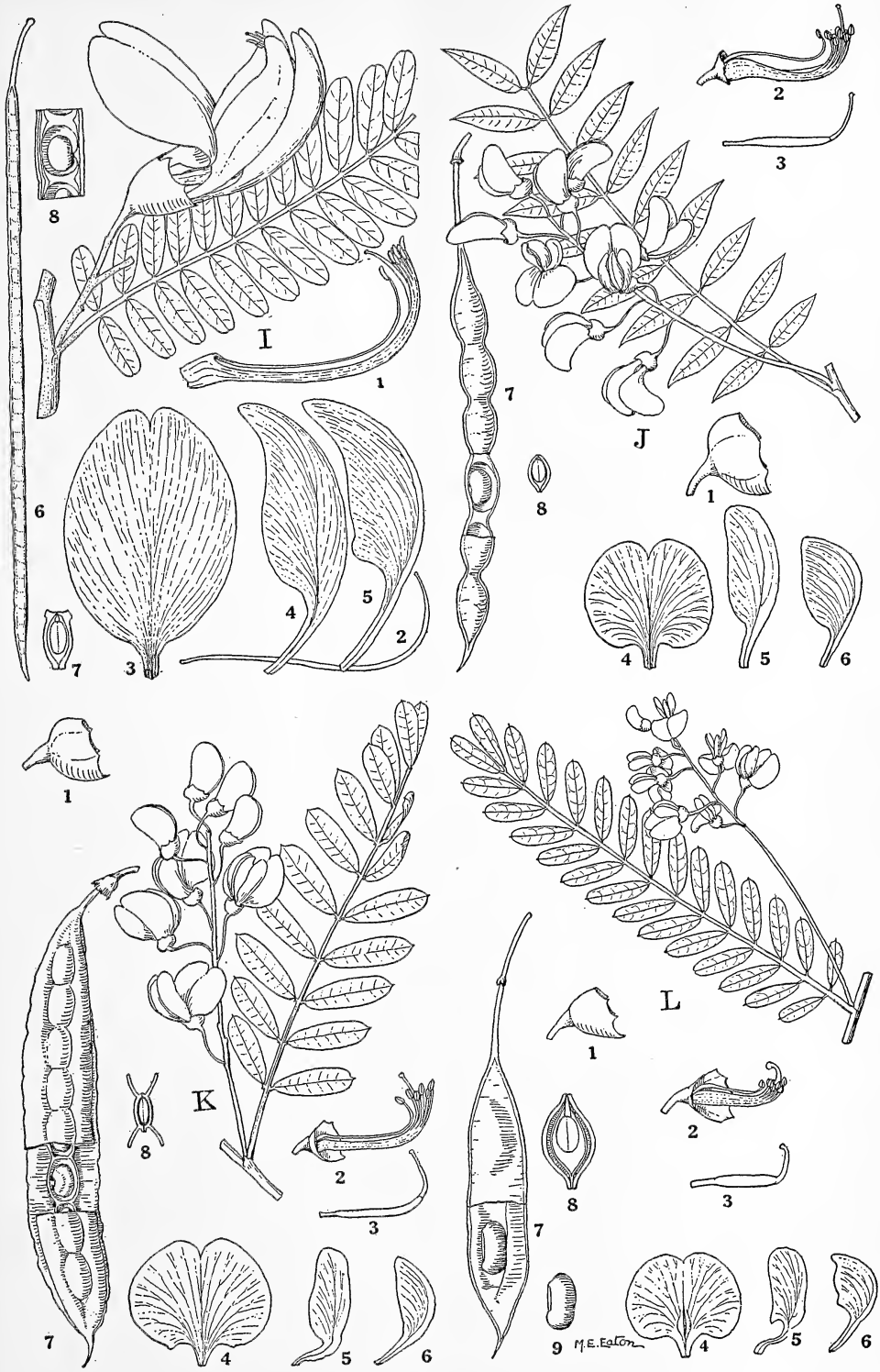


RYDBERG: NORTH AMERICAN FABACEAE

THE CAMBIUM AND ITS DERIVATIVE TISSUES IV. THE INCREASE IN GIRTH OF THE CAMBIUM

I. W. Bailey

(Received for publication December 19, 1922)

Introduction

Since the publication of Nägeli's (1864) "Dickenwachsthum des Stengels," most botanists, if we may judge from statements in standard textbooks, have assumed that the increase in girth of the lateral meristem, during successive stages in the enlargement of a stem or root, is due to "radial," anticlinal divisions of the cambial initials. Not all investigators, however, have accepted Nägeli's generalization. Robert Hartig (1895) inferred from the structure of the secondary xylem of *Pinus silvestris* L. that the increase in the periphery of the cambium in conifers is due primarily to the elongation of transversely dividing fusiform initials. Klinken (1914) reached a similar inference from the study of serial sections of the phloem of *Taxus baccata* L. He concluded that there are two fundamental types of meristematic activity, one characteristic of the conifers and the other of the dicotyledons. Neeff (1920) subsequently found evidences of Hartig's and Klinken's type of cambial activity in the xylem and phloem of *Tilia tomentosa* which led him to believe that there is no such fundamental distinction between the lateral meristems of gymnosperms and dicotyledons.

There is, of course, a considerable element of uncertainty in ascribing a particular type of meristematic activity to large groups of the vascular plants, either upon the basis of *a priori* deductions or upon that of indirect evidence obtained from the study of the xylem or phloem of one or two supposedly representative species.

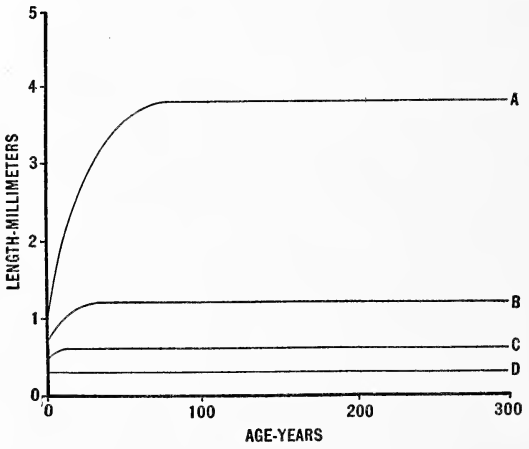
In 1917, the writer perfected methods for sectioning the cambium and for investigating its histological and cytological details. As stated in the third paper (1920 *b*) of this series, he did not succeed in finding evidences of the hypothetical radial, anticlinal divisions which are described and even figured in botanical textbooks. The normal, anticlinal divisions of the fusiform initials, in all of the gymnosperms and less highly specialized dicotyledons examined, were transverse or more or less oblique. That radial longitudinal divisions do occur, at least in certain cases, is suggested however by Kleinmann (1921), upon the basis of the orientation of karyokinetic figures in the cambium of Raphanus. In view of these facts, it is evident, on the one hand, that the increase in girth of the lateral meristem is not the simple phenomenon that Nägeli hypothesized, and, on the other

hand, that there may be more than one fundamental type of cambial activity in the vascular plants.

Analysis of the Problem

In Nägeli's formulae for computing the frequency of radial divisions, during a given increase in the diameter of a stem or root, the size of the cambial initials is treated as a constant. It is well known, however, that the cambium or lateral meristem is composed of initials of two distinct shapes and sizes: (1) *fusiform initials*, relatively large, elongated elements whose derivatives become differentiated into tracheids, fibers, vessels, sieve tubes, etc.; and (2) *ray initials*, scattered aggregations of small, more or less isodiametric cells which divide to form the horizontal sheets of radially disposed parenchyma, the so-called medullary rays (Plate XXXVI). Therefore, upon the basis of *a priori* considerations, the increase in girth of the lateral meristem might be due to one or more of the following factors:

1. An increase in the tangential diameter of the fusiform initials.
2. An increase in the length of these cells.
3. An increase in the number of these cells.
4. An increase in the diameter of the ray initials.
5. An increase in the number of these cells.



Text Fig. 1. Normal curves, showing average lengths of cambial initials at successive stages in the enlargement of a stem. *A*, Conifer or vesselless dicotyledon. *B*, Less specialized type of dicotyledon. *C*, Highly specialized type of dicotyledon. *D*, Highly specialized type of dicotyledon, having a stratified cambium.

In many of the vascular plants, as the writer has shown in previous papers of this series (1920, 1920*a*, 1920*b*), the initials tend to be larger in old stems than in young shoots, but they do not continue to increase in size throughout the entire life of an individual. Thus, in the conifers and

less specialized dicotyledons, where the fluctuations in cell size are considerable, the normal curve of average length of the fusiform initials at successive ages is of the general type illustrated in text figure 1. There is a rapid increase in length for a period of years until a certain size is attained which then remains more or less constant during succeeding growth of the plant. Are these variations in cell size significant factors in the increase in girth of the lateral meristem during the earlier stages in the enlargement of a stem? Some typical measurements taken from *Pinus Strobus* L. are of interest in this connection.

One-year-old stem

Radius of woody cylinder	2,000 microns
Circumference of cambium	12,566 microns
Average length of fusiform initials	870 microns
Average tangential diameter of fusiform initials	16 microns
Number of fusiform initials in a cross section of stem	724 microns
Average tangential diameter of ray initials	14 microns
Number of ray initials in a cross section of stem	70 microns

60-year-old stem

Radius of woody cylinder	200,000 microns
Circumference of cambium	1,256,640 microns
Average length of fusiform initials	4,000 microns
Average tangential diameter of fusiform initials	42 microns
Average tangential diameter of ray initials	17 microns

The increase in width of the original 724 fusiform initials would produce, during the 59-year interval, an arc of 30,408 microns, and the increase in the diameter of the 70 ray initials, during the same interval, an arc of 1,190 microns; or a total circumference of 31,598 microns as compared with the actual circumference of 1,256,640 microns. In other words, the enlargement of the cambial ring, during the 59-year interval, is due primarily to an increase in the number of initials; from 724 to 23,100 fusiform initials and from 70 to 8,796 ray initials.

It is to be emphasized in this connection that such a multiplication of cambial initials—*as seen in any given transverse plane or cross section of a stem*—is not due necessarily to cell division. For, if the initials elongate and slide by one another, the number which intersect a given transverse plane will be continually augmented. However, if the increase in the number of fusiform initials in *Pinus Strobus* were due entirely to longitudinal sliding growth, the original initials, during the 59-year interval, would have to attain an average length of approximately 26,800 microns instead of 4,000 microns. The ray initials do not elongate to any considerable extent.

In exceptional cases, *e.g.*, Sequoia, the fusiform initials may attain a maximum variability in length of 9,000 microns and in width of 60 microns,

but the rate of increase in size during a given period of years is not much in excess of the values recorded for *Pinus Strobus*. Furthermore, the ray initials in certain dicotyledons may have a maximum enlargement in diameter of from 30 to 40 microns, but the variability in the size of their fusiform initials is much less than that which occurs in most conifers.

It is evident, accordingly, that, although the increase in size of the cambial initials, during the earlier stages of the enlargement of certain plants, is by no means a negligible factor, the rapid increase in girth of the lateral meristem must in general be due largely to a progressive increase in the *number*, rather than in the *size*, of its constituent cells.

What then is the relative significance of the increase in the number of the two types of cambial initials? In the young shoot of *Pinus Strobus*, the combined diameters of the 70 ray initials form an arc of 980 microns, whereas those of the 8,796 ray initials in the 60-year-old stem constitute an arc of 149,532 microns, or approximately one eighth of the total circumference of the cambium. Many of the dicotyledons have a much higher percentage of ray initials. Indeed, in extreme cases more than one half of the circumference of the lateral meristem may be occupied by ray initials. Therefore, in discussing the *modus operandi* of the increase in girth of the cambium, it is essential to distinguish between the effects of (1) those anticlinal divisions which are concerned in the origin and multiplication of ray initials, and (2) those which produce an increase in the number of fusiform initials.

The reader should bear in mind in this connection that Nägeli's generalization is based upon the assumption that the divisions in both types of initials are radial, whereas the conclusions of Hartig, Klinken, and Neeff refer to the activity of the fusiform initials. The writer will likewise confine his attention in the following pages to the fusiform initials, reserving further discussion of the ray initials for a subsequent paper of this series.

Significance of the Arrangement of Fusiform Initials

In the gymnosperms and less specialized dicotyledons, the fusiform initials are not arranged in regular rows, whereas in certain of the more highly differentiated dicotyledons they are symmetrically grouped in parallel, horizontal series. The question suggests itself, accordingly, is this striking dissimilarity in the architecture of the lateral meristem indicative of fundamental differences in the growth and division of the fusiform initials, and, if so, what factors are concerned in the transitions from a non-stratified to a stratified arrangement?

As shown in figures 4–6, Plate XXXVI, the "fusiform" initials in stratified meristems are roughly hexangular with long parallel sides and abruptly tapering ends, and the elements of adjacent horizontal series do not overlap to any considerable extent. In other words, the form and the arrangement of the initials indicate very clearly that the increase in girth

of the cambium cannot be due to the *elongation* of transversely dividing cells; for, if it were, the superimposed initials must necessarily crowd by one another and ultimately break up the stratified arrangement. Conversely, if the anticlinal divisions are radio-longitudinal, the products of successive divisions should be grouped in horizontal rows, unless this arrangement is modified by differences in the elongation of adjacent elements. In non-stratified cambia, figures 1–3, the adjacent, overlapping fusiform initials vary considerably in length, but, as the writer has previously stated, the *average* length of these elements does not increase appreciably during the later stages of the enlargement of a stem or root. Thus, the increase in the number of fusiform initials in non-stratified meristems cannot be due solely to radio-longitudinal divisions; for, if it were, there would have to be a general increase in the length of the initials during all stages of the enlargement of the plant.

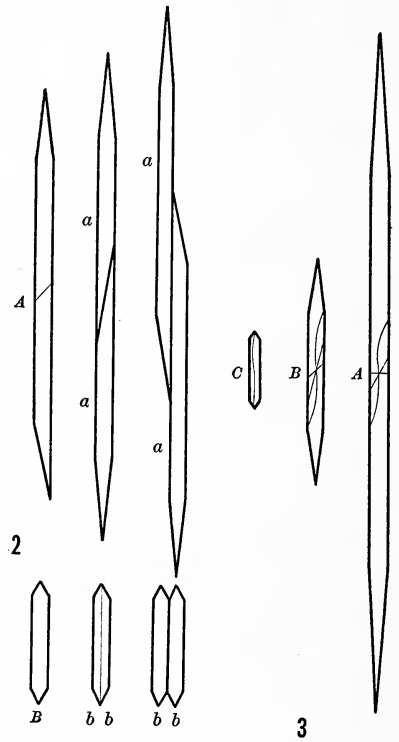
Such facts as these suggest that there are two fundamentally different types of meristematic activity—so far as the fusiform initials are concerned —in the vascular plants; *i.e.*, that the increase in girth of non-stratified cambia is due to the elongation of transversely dividing initials and that of stratified cambia to radio-longitudinal divisions of these elements. Detailed investigations of the cambium and of the histology of its derivative tissues, in numerous representatives of the gymnosperms and dicotyledons, strongly support such an assumption.

The writer has already referred to the fact that in all of the lateral meristems of conifers and less specialized dicotyledons investigated by him—which are, of course, of the non-stratified type—the anticlinal divisions are transverse or oblique (figures 8, 9). The orientation of the cell plates and recently formed anticlinal partitions in the stratified meristems of *Robinia Pseudo-Acacia* L. and of *Diospyros virginiana* L., on the contrary, is radio-longitudinal (figures 6, 7).

Furthermore, since the fusiform initials leave a record of their activities in the xylem and phloem, it is possible to trace successive stages of their growth and division in serial, tangential sections of these tissues.[1] Although this indirect method of studying cambial activity must be applied with a considerable degree of caution, particularly in the case of dicotyledons, it enables one to investigate a large number of plants of which suitable material of the lateral meristem is not readily available. In all of the various genera (62) of gymnosperms and dicotyledons that the writer has examined in this way, the evidence indicates very clearly that the anticlinal divisions in non-stratified meristems are pseudo-transverse, whereas those in stratified cambia are radio-longitudinal. In meristems of the former type, the fusiform initials elongate, sliding by one another, until they attain a certain size. They then divide by means of a more or less oblique partition into

[1] For detailed descriptions of this method, the reader is referred to Klinken's and Neeff's papers.

two short halves which in turn elongate and divide (text figure 2, *A*). Owing to the fact that the cells do not divide and elongate in unison, there is a very considerable variability in the length of adjacent initials. In addition, owing to the fact that the frequency of the anticlinal divisions and the elongation of the fusiform elements are not constant, the size of the initials tends to fluctuate more or less in different parts of a given individual; *e.g.*, they tend to be considerably shorter in slender shoots than in robust stems (text figure 1). Thus, the non-stratified arrangement and the variability in size of the fusiform cells are both due primarily to elongation



TEXT FIG. 2. Diagrams illustrating *modus operandi* of the increase in girth of cambium in non-stratified and stratified lateral meristems. *A*, Fusiform initial from non-stratified cambium, dividing pseudo-transversely; *a*, *a*, products of this division which elongate and slide by one another. *B*, Fusiform initial from stratified cambium; *b*, *b*, products of the radio-longitudinal division of this initial, which expand laterally but not longitudinally.

TEXT FIG. 3. Types of anticlinal divisions in fusiform initials. *A*, Typical fusiform initial of a conifer. *B*, Fusiform initial of a dicotyledon, having non-stratified cambium. *C*, Fusiform initial of a dicotyledon, having a stratified cambium.

following pseudo-transverse, anticlinal divisions. In meristems of the latter type, on the contrary, the initials divide radio-longitudinally and the products of such divisions expand laterally, but they do not elongate to any considerable extent (text figure 2, *B*). The structure of the secondary

tissues in the Calamariales, Sphenophyllales, Lepidophytineae, and Cycadofilices indicates that the cambia in these primitive groups of vascular plants were of the non-stratified type.

In view of these facts, we appear to be justified in concluding that there are at least two distinct, fundamental types of cambial activity in the vascular plants. In the vascular cryptogams, gymnosperms, and less specialized (structurally) dicotyledons, the anticlinal divisions are more or less transverse and the products of these divisions elongate and crowd by one another, producing thereby an increase in the girth of the cambium and a non-stratified arrangement of its cells. In certain of the more highly differentiated dicotyledons, on the other hand, the anticlinal divisions are radio-longitudinal and the products of these divisions expand laterally, thereby increasing the circumference of the cambium, but they do not elongate to any considerable extent, and thus become symmetrically grouped in parallel, horizontal series.

The Transition from the Non-stratified to the Stratified Arrangement

In previous papers of this series, the writer (1918, 1920*a*) has called attention to the fact that in the dicotyledons there is a progressive reduction in the length of the fusiform initials and of their derivatives, which closely parallels successive stages in the differentiation of highly specialized types of vascular tissues. As indicated in table 1, the vesselless dicotyledons (Tetracentron, Trochodendron, and Drimys), whose secondary xylem closely resembles that which occurs in the vascular cryptogams and gymnosperms, have large fusiform initials; fully as large as those of most gymnosperms, for example. Dicotyledons with vessels, on the contrary, are characterized by having much smaller meristematic cells which become shorter and shorter as the tracheary elements become more and more highly specialized. Furthermore, stratified meristems tend, in general, to be composed of smaller fusiform initials than non-stratified cambia. It should be noted, in addition, that, during this sequence of changes, the size-on-age curves (text figure 1) are depressed and ultimately become approximately horizontal; *i.e.*, the length of the fusiform initials is stabilized in plants having stratified meristems.

These facts are of considerable interest in discussing the *modus operandi* of the transitions from one fundamental type of meristematic activity to the other. In most of the non-stratified meristems that the writer has studied, the orientation of the anticlinal partitions fluctuates between a transverse position and varying degrees of obliquity (text figure 3). As the fusiform initials become shorter, the ends of the more oblique partitions tend to approach the extremities of the cells, or, in other words, to become more and more nearly radio-longitudinal. Thus, certain of the more highly differentiated dicotyledons have transitional types of meristems, which show in-

TABLE 1. *Length (in microns) of adjacent fusiform initials in random samples of the lateral meristem of old stems*

NON-STRATIFIED CAMBIA

Gymnosperms

Species	Max.	Ave.	Min.
Ginkgo biloba L.	3,000	2,200	1,400
Pinus Strobus L.	4,000	3,200	2,300
Picea Abies (L.) Karst.	4,200	3,300	2,400
Juniperus virginiana L.	3,000	2,200	1,000
Larix decidua Mill.	5,000	4,000	2,500
Sequoia sempervirens Endl.	8,700	6,600	4,200
Agathis robusta F. M. Bailey	7,700	6,800	4,100
Tsuga canadensis (L.) Carr.	4,400	3,200	2,200
Podocarpus Nageia R. Br.	5,000	3,800	2,300
Cedrus libani Barrel	4,100	2,900	2,100
Average	4,910	3,820	2,450

Dicotyledons

A. Vesselless

Trochodendron aralioides Sieb. et Zucc.	6,200	4,400	2,800
Drimys Winteri Forst.	4,500	3,300	2,400
Average	5,350	3,850	2,600

B. Vascular tissues not highly differentiated

Betula populifolia Marsh.	1,160	940	700
Myristica philippensis Lam.	1,620	1,310	990
Altingia excelsa Noronha	2,300	1,900	1,200
Liriodendron tulipifera L.	1,500	1,100	700
Urandra luzoniensis Merr.	1,700	1,400	1,100
Dillenia philippinensis Rolfe	2,300	1,600	1,000
Gordonia Lasianthus L.	1,700	1,300	1,000
Cornus florida L.	1,400	1,100	800
Symplocos tinctoria L'Hér.	1,400	1,100	600
Halesia diptera L.	1,100	900	800
Average	1,620	1,260	890

C. Vascular tissues highly specialized

Carya ovata (Mill.) C. Koch	600	520	420
Litsea glutinosa C. R. Rob.	700	550	390
Prunus serotina Ehrh.	590	460	320
Excoecaria Agallocha L.	870	630	410
Mangifera monandra Merr.	830	570	390
Acer rubrum L.	610	490	320
Garcenia dulcis Kurz.	1,020	740	520
Vatica Mangachapoi Blanco	810	610	410
Barringtonia racemosa (L.) Roxb.	900	720	500
Psychotria luzoniensis F. Vill.	1,080	700	450
Average	800	600	410

STRATIFIED CAMBIA

Dicotyledons

D. Vascular tissues highly specialized

Species	Max.	Ave.	Min.
Grewia multiflora Juss.	370	250	160
Thespesia populnea (L.) Soland. ex Corr.	280	250	210
Bombycidendron Vidalianum Merr. et Rolfe	430	360	320
Heritiera littoralis Dryand.	360	300	270
Kleinhovia hospita L.	480	360	270
Pterospermum niveum Vid.	430	370	320
Tarrietia sylvatica Merr.	340	280	210
Sterculia foetida L.	450	370	320
Robinia Pseudo-Acacia L.	210	170	140
Diospyros virginiana L.	520	410	320
Average.	390	310	250

Basis: 50 measurements of fusiform initials.
Probable errors of individual averages 10–15 percent.

cipient stages of stratification. Furthermore, the variability in the size of adjacent fusiform initials in stratified meristems (table 1) is due, at least in part, to the fact that many of the anticlinal divisions are somewhat oblique.[2]

How much significance should be attached to the close parallelism in the sequences of changes in the cambium and vascular tissues? Is the progressive reduction in cell size in the lateral meristem due to the increasing specialization of the vascular tissues, or *vice versa?* Many morphologists interpret such correlations as due to cause and effect. There is, however, a very considerable element of uncertainty in so doing, where the basis of comparison is time, as Karl Pearson has so clearly shown. Thus, in the absence of reliable collateral evidence it is not possible to determine whether the changes in the cambium are due to those which occur in the vascular tissues or *vice versa*, or whether the parallel sequences are both due to some third factor or group of factors.

SUMMARY AND CONCLUSIONS

1. There are two fundamental types of cambial activity in the vascular plants: one characteristic of the Calamariales, Sphenophyllales, Lepidophytineae, Cycadofilices, Gymnospermae, and less differentiated (structurally) Dicotyledoneae, and the other of certain highly specialized dicotyledons.

2. In the former type the fusiform initials are not arranged in regular rows. The anticlinal divisions are pseudo-transverse and the products of

[2] The size and the arrangement of the fusiform initials is also modified by the ray initials, new aggregations of which are periodically carved out of the elongated elements.

these divisions elongate and crowd by one another, thereby producing an increase in the girth of the cambium.

3. In the latter type, in which the fusiform initials are relatively short, of nearly uniform length, and more or less symmetrically grouped in parallel, horizontal series, the bulk of the anticlinal divisions is radio-longitudinal, and the increase in the periphery of the cambium is due primarily to the lateral expansion of the products of these divisions.

4. The transition from the non-stratified to the stratified arrangement closely parallels successive stages in the specialization of the vascular tissues, *e.g.*, the differentiation of vessels, libriform fibers, etc., and appears to be due to a progressive reduction of cell size and of longitudinal sliding growth in the cambium.

Acknowledgments

The writer is much indebted to Doctor E. D. Merrill, Director of the Philippine Bureau of Science, and to Professor I. F. Lewis for their kindness in sending freshly killed material of a number of different plants.

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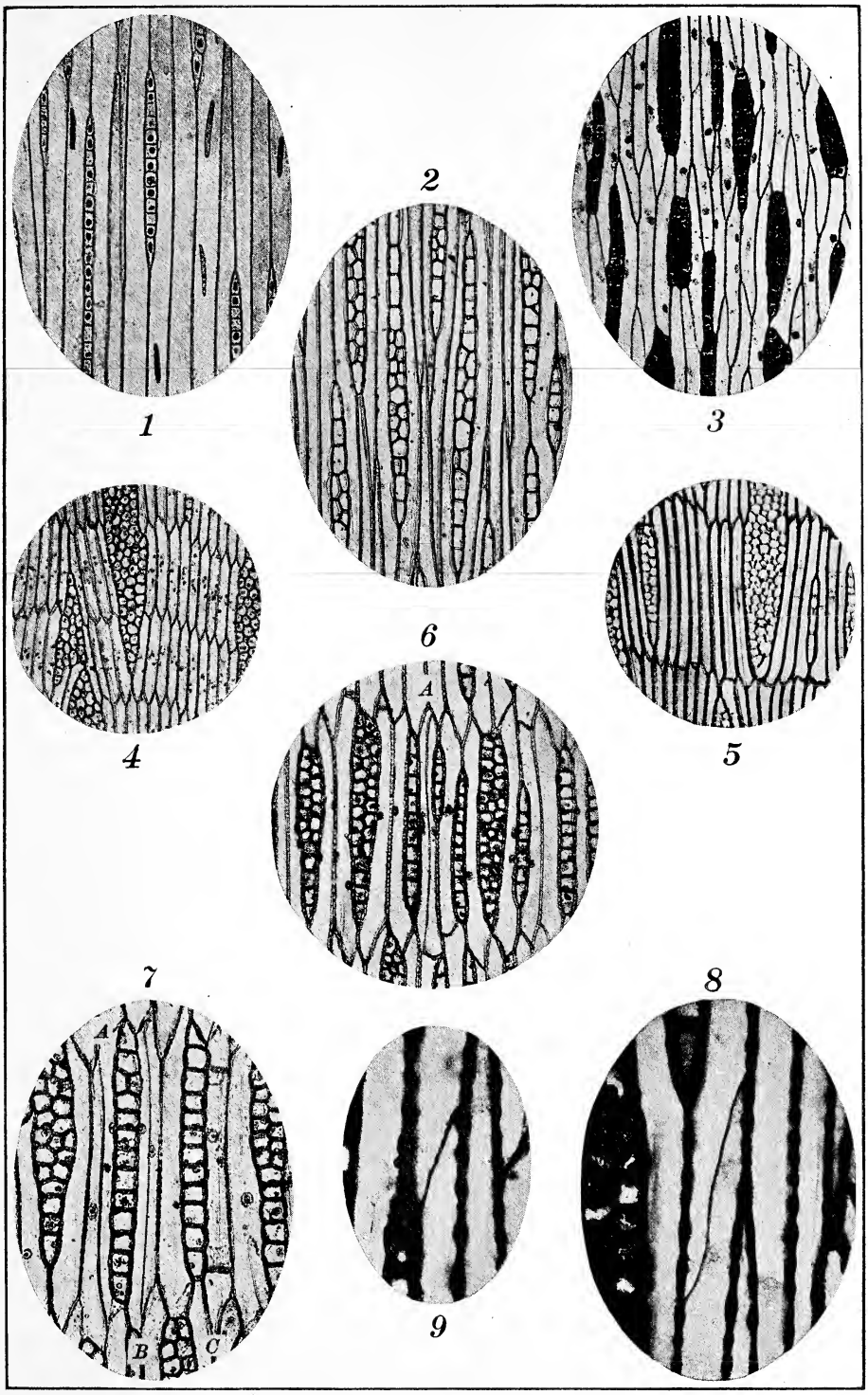
EXPLANATION OF PLATE XXXVI

FIG. 1. *Pinus Strobus* L. Tangential longitudinal section of non-stratified lateral meristem. The cells are so long that only a portion of each fusiform initial is shown in the photomicrograph. × 110.

FIG. 2. *Myristica philippensis* Lam. Tangential longitudinal section of non-stratified lateral meristem of less highly differentiated type of dicotyledon. × 110.

FIG. 3. *Fraxinus americana* L. Tangential longitudinal section of non-stratified lateral meristem of highly specialized type of dicotyledon. × 110.



BAILEY: CAMBIUM AND DERIVATIVE TISSUES

FIG. 4. *Robinia Pseudo-Acacia* L. Tangential longitudinal section of stratified lateral meristem of highly specialized type of dicotyledon, showing parallel horizontal series of fusiform initials. × 110.

FIG. 5. *Heritiera littoralis* Dryand. Tangential longitudinal section of immature phloem, showing stratified elements. × 110.

FIG. 6. *Diospyros virginiana* L. Tangential longitudinal section of stratified lateral meristem. Fusiform initial (*A*) has divided radio-longitudinally. × 110.

FIG. 7. *Same.* Portion of cambium more highly magnified. Cells *A* and *B* have formed radio-longitudinal, anticlinal partitions. Cell *C* is dividing periclinally. The kinoplasmasomes are approaching the ends of the cell. × 148.

FIG. 8. *Liquidambar Styraciflua* L. Tangential longitudinal section of non-stratified lateral meristem, showing oblique anticlinal division of fusiform initial. × 450.

FIG. 9. *Same.* Oblique anticlinal division. × 450.

TYPE SPECIES OF THE FIRST 100 GENERA OF LINNAEUS' SPECIES PLANTARUM

A. S. Hitchcock

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The type concept is finding increasing favor with botanists everywhere. So far as its application to future cases is concerned it may be regarded as already accepted. But when it comes to applying the principle retroactively there is some hesitation on the part of many. Because of the way in which it has been applied by some of the adherents of the American Code, there has been a fear that many well-established names might be superseded if the method were generally used. The rules of the Type-basis Code are more elastic than those of the American Code and would permit a reasonable application of the principle without introducing unnecessary confusion. In my account of the Genera of the Grasses of the United States[1] I typified about 300 genera of grasses according to the rules set forth in the Type-basis Code. In order to test the effect of applying these rules to other genera of flowering plants, I have tentatively typified the first 100 genera of Linnaeus' Species Plantarum. The results are here set forth and analyzed.

Of the 100 genera, 28 contain a single species. These are the types of the respective genera according to any set of rules. Most botanists agree that a generic name should be applied so as to include its original species when there is only one, and one of its original species where there are more than one. However, there are a few cases in which the historic development has followed a different course. An application of the type concept may here cause a profound dislocation of names if a large number of species are involved. Such cases should be considered on their merits and exceptions made if it seems worth while and if a general agreement can be reached.

Among the genera mentioned (often called monotypic genera), there are two cases in which the original species is not included in the genus as now generally accepted. Since the original species is in each case the type, an application of the type concept necessitates a readjustment of the nomenclature of each group. *Alpinia racemosa*, the single species of Alpinia, is now referred to Renealmia, though the genus Alpinia, containing a large number of species, is recognized as valid. Dr. E. D. Merrill has called attention to this case. Among the genera of group 1 there is one other case of this kind, *Eranthemum capense*, referred to Daedalacanthus, though the genus Eranthemum with many species is accepted as valid.

[1] U. S. Dept. Agr. Bull. 772. 1920.

The remaining 72 genera, containing originally more than one species, must be typified by selecting one of the species as the type. It is this retroactive application of the type concept that has aroused the most opposition from the adherents of the International Rules because of the fear that well-established names would be needlessly displaced. I think I can demonstrate that the results are not revolutionary and that in the main the fears are groundless. A uniform application of any set of rules will bring to light certain anomalous cases which in some way should be considered and either corrected or validated.

One of the first things to do in selecting a type is to exclude from consideration those species that definitely disagree with the generic description, because certainly an author would not illustrate or typify his own genus with an anomalous species. As there are no generic descriptions in the Species Plantarum, the descriptions in the fifth edition of the Genera Plantarum (1754) are used to ascertain Linnaeus' concept of the genera included in the Species Plantarum. The typification on the basis of the generic description should be done only by those familiar with the taxonomy of the groups. I have not attempted to do this except with the grass genera. As an illustration, we have the genus Holcus which does not occur in the first 100 genera now under consideration. The generic description in the Genera Plantarum certainly applies only to three of the seven species included in the Species Plantarum, the three species related to *Holcus Sorghum* which were later segregated as the genus Sorghum. The application of the type method here goes contrary to historical development subsequent to Linnaeus and to the general usage of those who recognize the group as distinct from Andropogon. In current usage the genus is represented by *Holcus lanatus*.

It is rather exceptional among Linnaean genera to find species definitely excluded in this way from consideration in selecting the type. It is possible that on taxonomic grounds there may be a few changes in the tentative list of type species here presented.

The next point to ascertain is which species the author of the genus appeared to have chiefly in mind, in so far as one species can be singled out. We may assume, unless there is evidence to the contrary, that the representative species to the author would be the one best known to him. This may be shown in four ways: First, one to which he has applied a specific name like *officinalis*, *communis*, *vulgaris*, or *sativus;* second, a well-known economic species; third, a common species of the native flora or one grown by him in a garden; fourth, through a citation in the Genera Plantarum. Another method, the selection on the basis of figures accompanying the original description, can not be used here because there are no plates in the Species Plantarum. These four methods are used coördinately. Sometimes one can be applied, sometimes another. Often two or more methods lead in the same direction, as in Hordeum, of which *H. vulgare* is selected as the

type on the basis of its being an economic species. But it would be indicated as the type through the name *vulgare* and through the Tournefort figure cited in the Genera Plantarum. If different methods conflict, the factors must be considered and a balance struck. In the list submitted there appears to be no conflict except in Justicia which is considered separately.

In selecting a native species one assumes that a European species will be better known to Linnaeus than one from some other continent; that a Swedish species would be better known than one from southern Europe; and that one grown in the Hortus Cliffortianus or Hortus Upsaliensis better known than one represented by a herbarium specimen only. One should select a species on the basis of a figure cited in the Genera Plantarum only when there is no doubt as to the identity of the figure, and the method should be used with caution.

After these four methods have been applied there are still some cases in which a selection has not resulted. At this point the historical development should be considered. In these genera, amounting to about 20 percent in our list, the type has been chosen from among the original species now commonly retained in the genus, thus fixing the application of the generic name in accord with current usage.

In case there are more than one residual species, the type is the most common or best known, or, if equally eligible, the first of these.

Among the 72 genera considered there are a few that must receive attention separately.

Justicia contains 9 species of which four are retained in the genus under present usage. The first of these is *J. betonica*. The citation in the Genera Plantarum refers to *J. sexangularis*, which is now usually placed in Dicliptera. The selection of this species as the type would change the application of Justicia as currently understood. If the citation in the Genera Plantarum is ignored, the type is *J. betonica* and the genus falls in group 5.

Ixia contains two species, both of which are now referred to later genera, *I. africana* to Aristaea, *I. chinensis* to Belamcanda. On the type basis one of these species, probably the first, should be accepted as the type, and the nomenclature of the other groups adjusted accordingly.

Minuartia contains 3 species, all of which are currently referred to Alsine.[2] The nomenclature of this group has been considered by Sprague and others. The three species, all from Spain, appear to be equally eligible for the type, and the first, *M. dichotoma*, may be selected.

Aira contains 14 species of which four were included in the first use of the name in the Flora Lapponica. The type would ordinarily be chosen

[2] In the original issue of the Species Plantarum, Minuartia appears with a single species, *M. hispanica*. This leaf (pages 89 and 90) was reprinted and inserted in place of the original. A very few copies escaped the correction. An account of the insertion of the corrections is given in Bot. Centralbl. **66**: 216. 1896, **67**: 5. 1896, and Jour. Bot. **34**: 359. 1896. The photographic reprint of the work was made from the original issue.

from these four as they represent Linnaeus' original concept of the genus and there is nothing to show that the concept was altered in the Species Plantarum, except by enlargement. Of these four, one, *A. spicata*, is now referred to Trisetum. In my account of the Genera of Grasses I selected *A. caespitosa* as the type. The historic development was different. The last-mentioned species was taken out as the type of Deschampsia and the other species were referred to other genera, leaving in Aira, as commonly accepted, only *A. praecox* and *A. caryophyllea*, species not found in the Flora Lapponica. In this case my application of the type method gives a result contrary to current usage.

Leucadendron contains 13 original species, and Protea contains two. In the Index Kewensis all the original species of Leucadendron are referred to Protea, and the two species of Protea are referred to Leucadendron. Both genera are accepted as valid, but neither, as accepted, contains any of its original species. An application of the type method will seriously disturb two large genera. Leucadendron and Protea are included in the list of conserved names of the International Rules.

The purpose has been to show that the rules as given in the Type-basis Code for the typification of genera when concretely applied do not result in any startling upheaval of nomenclature or radical changes in the application of generic names. The few cases mentioned at the end, only six percent of the list, would require special attention under any set of rules.

The 72 genera in which there are more than one original species are grouped below, each with its type species as selected according to the method outlined. The first four groups are coördinate and the sequence has no significance.

Group 1. Specific name *officinalis*, *vulgaris*, *communis*, or *sativus*.

Jasminum officinale
Syringa vulgaris
Veronica officinalis
Gratiola officinalis
Pinguicula vulgaris
Utricularia vulgaris
Verbena officinalis
Salvia officinalis
Valeriana officinalis
Crocus sativus
Gladiolus communis
Commelina communis

Group 2. The type an economic species.

Piper nigrum
Saccharum officinarum
Panicum miliaceum
Phleum pratense
Alopecurus pratensis
Agrostis stolonifera
Poa pratensis
Dactylis glomerata
Avena sativa
Lolium perenne
Secale cereale
Hordeum vulgare
Triticum aestivum[3]

[3] *Triticum aestivum* and *T. hybernum* are equally eligible.

Group 3. Type the commonest or best-known species.

Kaempfera galanga
Boerhavia diffusa
Salicornia europaea
Olea europaea
Lycopus europaeus
Milium effusum
Melica nutans
Briza media
Cynosurus cristatus
Festuca ovina
Elymus sibiricus
Halosteum umbellatum
Mollugo verticillata
Queria hispanica
Blitum capitatum

Group 4. Type based on the citation in the Genera Plantarum.

Canna indica
Phyllyrea latifolia
Iris germanica
Cyperus rotundus
Scirpus lacustris
Bromus secalinus
Eriocaulon decangulare

Group 5. One of the residual species.

Amomum cardamon
Curcuma longa
Corispermum hyssopifolium
Nyctanthes arbor-tristis
Chionanthus virginicus
Circaea lutetiana
Zizophora capitata
Monarda fistulosa
Anthoxanthum odoratum
Antholyza cunonia
Schoenus nigricans
Eriophorum vaginatum
Nardus stricta
Phalaris canariensis
Uniola paniculata
Lechea minor
Cephalanthus occidentalis
Stipa pennata
Arundo donax

Summary

Genera with a single original species	28
Genera with more than one original species	72
Group 1. Type based on specific name	12
Group 2. Type an economic species	13
Group 3. Type a well-known species	15
Group 4. Type based on citation in Genera Plantarum	7
Group 5. Type a residual species	19
Special cases	6
Total	100

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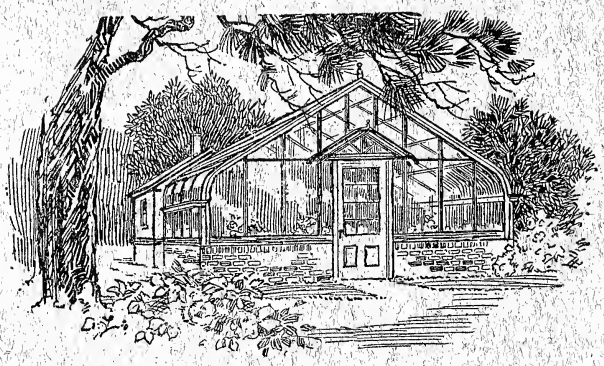
AMERICAN JOURNAL OF BOTANY.

June 1, 1923.



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AUSTRALASIAN BOTANICAL NOTES
III. NEW ZEALAND

DOUGLAS HOUGHTON CAMPBELL

(Received for publication January 2, 1923)

Although one is accustomed to associate New Zealand and Australia, as divisions of Australasia, the two countries nevertheless differ much from each other both in climate and topography, and these differences are reflected in the character of the vegetation. New Zealand, on the whole, differs markedly from Australia in the general aspect of its flora. It is not always realized that the two countries are sundered by more than a thousand miles of sea—much too great a barrier to admit of any considerable interchange of plants under existing conditions. To the north of New Zealand, but separated by about the same distance as that to Australia, lie Fiji, Tonga, and Samoa, but no large land-masses occur in the great Pacific area north of New Zealand.

New Zealand consists of two large islands, the North and South Islands, and a number of much smaller outlying ones, comprising altogether 104,471 square miles. Of this area all but about 500 square miles belongs to the two main islands, the South Island being somewhat larger than the North Island. These two islands occupy about twelve degrees of latitude (34° –46°), while the Kermadecs to the North reach 30°, and the sub-antarctic islands to the south extend to 55°. This of course means a considerable difference in temperatures between north and south, but not so much as might be inferred, owing to the decidedly insular climate which prevails throughout New Zealand, as no part of the country is more than 100 miles from the sea and the coast-line is very extensive, with innumerable indentations often reaching far inland.

Unlike Australia, New Zealand is extremely mountainous, and the mountains are much higher than those of Australia. These lofty mountains exercise a powerful influence on the rainfall which is extremely heavy in certain districts, and, except in a few localities, is generally abundant and well distributed. As a whole, the climate is a distinctly temperate one. At the north, frost is practically absent, except at higher elevations, and even in the extreme south the winters are mild with little severe freezing weather.

[The **Journal** for November (**10**: 459–514) was issued November 27, 1923.]

As might be expected from its great isolation, New Zealand, like Australia, has a very high percentage of endemic species. According to Cockayne,[1] of the vascular plants 74 percent are endemic, and, if only seed-plants are considered, 79 percent. Of the dicotyledons, no less than 85 percent are peculiar.

The mean annual temperature of Auckland, in the North Island, is 59.2° F. (February, 67.1°–July, 51°); Invercargill, in the extreme south of the South Island, 10° farther south, has a mean temperature of 50° (February, 56.6°–July, 41.2°).

The rainfall is largely controlled by the position of the mountain ranges. The prevailing winds are westerly, and the west coasts receive a maximum rainfall; but in the South Island, especially, the extensive lofty range of the Southern Alps, parallel to the West Coast, intercepts a large amount of the moisture, so that, while stations to the west may have 200 inches of rain annually, eastern stations like Christchurch may show only 25 inches, or even less. The average throughout New Zealand is 40–50 inches, with a pretty even distribution through the year, although the autumn and winter are somewhat wetter, and the summer is the dryest season.

The main range of mountains in the North Island extends from the extreme south to about the middle, following the east coast, but much lower than the Southern Alps, the highest peaks being under 1800 meters.[2] The loftiest mountains of the North Island are volcanoes arising from a plateau in the center of the island—the Rotorua district celebrated for its volcanoes, geysers, and hot springs—a region familiar to tourists visiting New Zealand. Ruapehu, 2803 meters elevation, is the highest mountain of the North Island, and its summit is permanently snowclad. Some small glaciers also occur. Mt. Egmont, on the west coast, is a fine volcanic cone 2514 meters in height, and also crowned with perpetual snow. In general, the surface of the North Island is very rugged, with relatively little level country. The most extensive level region is the Wanganui Plain, sloping gradually from the western coast to Mt. Ruapehu. Streams are abundant, but none of the rivers are of great length, owing to the limited size of the island. Lakes are not infrequent, the largest being Lake Taupo, on the volcanic plateau.

The South Island is not only larger than the North Island, but the mountains are much higher. The Southern Alps extend for nearly the whole length of the island parallel with the west coast, and culminate in Mt. Cook, 3766 meters. Both because of the greater elevation and of the higher latitude, these mountains are permanently snow-clad, and from them, in the southern portion, descend great glaciers reaching almost to sea level. The Franz Josef glacier terminates at 218 meters above the sea. To the east of the Alps are extensive plains, the most important being the Canterbury Plain, 48 kilometers across in its widest part. To the west of the moun-

[1] Cockayne, L. Vegetation of New Zealand. Die Vegetation der Erde 14: 311: 1921.

[2] Cockayne, *loc. cit.*, p. 23.

tains is the narrow Westland plain, a strip about 10 kilometers wide between the mountains and the sea. The sea-coast is extremely irregular, with many deep inlets. In the southwest part of the island, the numerous fiords offer some of the finest scenic features of New Zealand.

As we have already seen, the flora of New Zealand is highly peculiar; but, on account of the relatively small area of the country and of its uniform climate, it is much less extensive than that of Australia, and there are no such contrasts as, for instance, that between the floras of tropical Queensland and of West Australia. Cook's Strait has very little effect as a barrier, and many species extend practically throughout the whole extent of both North and South Islands.

An analysis of the flora shows that in spite of its temperate climate and southward extension, the Malayan element is predominant. While certain Australian families, genera, and even species occur, they are by no means so abundant as those of unmistakable Malayan origin, and, moreover, many of the genera which New Zealand shares with Australia are Malayan types, rather than truly Australian; *e.g.*, Agathis, Metrosideros, Dendrobium, and many ferns. The South American, or "Fuegian" element, which is also represented to some extent in Australia (especially in Tasmania), is an important factor in New Zealand, especially in the South Island, but well represented also in the mountain floras of the North Island.

In the North Island especially, but to some extent also in the South Island, the luxuriant forests which covered much of the country have been ruthlessly swept away. While this has been done to some extent for timber, a much larger area has been destroyed to provide pasturage for the flocks and herds which constitute the main wealth of the country. The loss of timber, and the damage due to denudation of water-sheds, must be very great; and large stretches of waste country covered with bracken fern and worthless scrub have replaced the vanished forests all over the country. As one looks at these worse than useless wastes, one can not but feel that the pastoral industries have been a bit overdone in Australasia.

Originally the North Island was very largely covered with heavy forest, in much of which the Kauri pine (*Agathis Australis*) was the most important tree. Owing to the great value of its timber, very little remains of this largest and most valuable timber tree in New Zealand. A tract of fine Kauri forest north of Auckland has been acquired by the government as a national park, and I had an opportunity of visiting this, which gives an excellent idea of the great forest which formerly covered the adjacent country.

As one approaches the Kauri forest, the huge spreading crowns of the giant trees are seen standing far above the other trees, reminding one, in a way, of the lofty domes of the sequoias overtopping the other trees of the Sierra forest. The habit of the Kauri, however, is very different from that of any of our great American conifers. The leaves are broad and in shape

like an olive leaf, while the tree, except when young, hardly suggests a conifer. At first it has a symmetrical conical growth like a pine or fir; but after a time, the lateral branches fall off, leaving a smooth cylindrical bole with very little taper. This columnar trunk may reach a height of 60–80 feet, or possibly more, and then divides into several great diverging branches which form an immense spreading crown of foliage, giving the tree a most characteristic appearance.

TEXT FIG. 1. Base of Kauri (*Agathis Australis*). North Auckland district. Photographed by Dr. L. Cockayne.

The interior of the Kauri forest is most impressive. At intervals the huge gray columns rise—sometimes 8 or 10 feet or even more in diameter, and between them are the slender stems of smaller trees. The ground is covered with a carpet of vegetation—ferns, sedges, liverworts, and mosses, among which grow young trees and shrubs of various species. The largest tree I saw measured 36 feet, 6 inches in circumference at about six feet above the great heap of humus at its base. It is said that trunks upwards of 20 feet in diameter have been measured, but it is doubtful if any trees of these dimensions have survived the onslaughts of the lumbermen. In any case, the Kauri must be classed with the giants of the Vegetable Kingdom.

The commonest tree associated with the Kauri is *Beilschmiedia taraire* (Lauraceae), a slender tree about 50 feet high. Another common and conspicuous tree is the "rata" (*Metrosideros robusta*), very showy when covered with its crimson flowers, much like those of the Hawaiian *M. polymorpha*.

There are also several gymnospermous trees, *viz.*, *Podocarpus totara*, *P. ferruginea*, *Dacrydium cupressinum*, and *Phyllocladus trichomanioides*, the latter a small tree with peculiar flattened, leaf-like twigs ("cladodes") looking like fern leaves.

Where the forest is not too dense, there is a heavy growth of vegetation on the forest floor—huge tussocks of a coarse sedge (*Gahinia xanthocarpa*), and a somewhat similar plant, *Astelia trinervis*, of the Liliaceae, are common and conspicuous; ferns in great variety are seen on every side. Characteristic ferns of the Kauri forest are *Blechnum Fraseri* and *Dicksonia lanata*, large and handsome species, and the beautiful tree-fern *Cyathea dealbata*, easily recognized by the silvery under side of the leaves. The filmy ferns (Hymenophyllaceae) are common, but less noticeable than in the very wet rain-forest of the South Island.

There are many fine shrubs, especially in the more open places. Among these *Coprosma grandifolia*, *Panax arboreum*, and *Myrtus bullata* are conspicuous, and in some places the magnificent tree-fern *Cyathea medullaris*.

The only palm native to the large islands of New Zealand is the "Nikau" (*Rhopalostylis sapida*), which is very abundant in the Kauri district. The smooth slender stem seldom exceeds 20 feet in height, and the stiff pinnate leaves form an upright tuft on the top.

Lianas are not abundant in the Kauri forest. The climbing fern, *Lygodium articulatum*, is not uncommon but confines itself to the smaller trees and shrubs. *Freycinetia banksii* is also common, but does not always assume a climbing habit.

Epiphytes are common on the smaller trees, but the smooth trunk of the Kauri does not afford a favorable foothold for most epiphytes. *Metrosideros robusta*, which itself usually begins life as an epiphyte, is especially favored by other epiphytic growths, and the Nikau palm is a favorite host for one of the most conspicuous epiphytes, *Astelia Solanderi*, which forms great clumps of sword-shaped leaves, often completely encircling the slender trunk of the palm. A good many ferns are epiphytes, *e.g.*, most Hymenophyllaceae, *Blechnum filiforme*, several species of Polypodium and Asplenium; and of course there are many mosses, liverworts, and lichens. Epiphytic orchids are much less abundant than in the Australian rain-forest, and are all inconspicuous species. Perhaps the commonest is *Dendrobium Cunninghamii*.

Among the interesting ferns of the North Island may be mentioned the following; *Todea barbara*, *Schizaea dichotoma*, *Gleichenia circinnata*, *G. dicarpa*.

Where the forest has been cut away, two species of Leptospermum, *L. scoparium* and *L. ericoides*, quickly take possession of the cleared areas. This "manuka" scrub is a familiar feature of the New Zealand landscape.

In the wetter open ground two other very characteristic plants cannot fail to attract attention. The "flax," *Phormium tenax*, often covers exten-

sive tracts with its clumps of tall, stiff leaves, five or six feet high, and at the time of my visit the flowering scapes, almost twice the height of the leaves, were opening their racemes of dark-red tubular flowers, attracting the honey-eating birds. Associated with the flax is the "cabbage-tree," *Cordyline Australis*, much resembling a tree-Yucca, and often grown in California under the name "Yucca-palm." It bears great panicles of small very fragrant white flowers. These two plants in full flower, together with the extensive thickets of Leptospermum covered with myriads of pretty white blossoms, made a very attractive floral display.

In the moist, sheltered gullies, a quite different type of vegetation prevailed. These gullies were quite filled with a dense growth of tree-ferns and palms, presenting a quite tropical picture and a great contrast to the dry manuka scrub.

TEXT FIG. 2. Tussock-grass formation; in background, *Cordyline Australis* and *Phormium tenax*. Photographed by Mr. W. D. Reid.

In the vicinity of Auckland are several very perfect small volcanic cones, the most interesting being Rangitoto, an island in the harbor a few miles from Auckland. This has been set aside as a reservation, and the native vegetation has been very little interfered with, and, as it is several miles away from the mainland, only a few weeds have obtained a foothold. The

cone is about 300 meters in height, and the ascent is a very easy one. At the summit is a perfect crater, at the bottom of which a solitary pine was noticed—probably a seedling of the Monterey pine (*P. radiata*). This species is the commonest tree in cultivation in New Zealand, and the seeds are readily dispersed by the wind. A few weeds, also obviously migrants from the mainland, were noted, and a few plants of the common fox-glove—also unmistakably introduced.

Dr. D. Petrie, of Auckland, a well known student of the New Zealand flora, was in our little party, and pointed out the many interesting plants comprising the flora of the island.

The slopes of the volcano are composed of broken masses of scoria, and growing on these it was interesting to note a number of species which ordinarily are rain-forest epiphytes, such as *Peperomia Endlicheri*. Perhaps the most notable of these were *Metrosideros robusta* and *Griselinia lucida*. Among the most unexpected denizens of these exposed volcanic rocks were two or three filmy ferns, one of which, *Trichomanes reniforme*, is a common rain-forest epiphyte. The behavior of these plants recalls the similar habit of certain Hawaiian species which invade the recent lava flows. In Hawaii, *Metrosideros polymorpha*, usually, like *M. robusta*, an epiphyte in its juvenile stage, is one of the first trees to invade the bare lava.

Other rain-forest species, *e.g.*, *Coprosma lucida*, *C. robusta*, and *Geniostoma ligustrifolium* manage to grow, but have developed thicker and smaller leaves than in their usual habitat.[3]

Among the other trees and shrubs, a second species of Metrosideros, *M. tomentosa*, was conspicuous. This is abundant in the Auckland district, and is said to be very beautiful about Christmas time when covered with masses of crimson flowers. Both species of Leptospermum were seen, and the brilliant green foliage of the Coprosmas and Geniostoma, already mentioned, at once caught the eye, as did a handsome Araliad, *Panax arboreum*, a common and beautiful shrub or small tree. My attention was also called to a number of less striking and less familiar genera—*e.g.*, Myrsine, Dodonaea, Pomaderris, Myoporum, and others.

A number of genera which in northern latitudes are usually herbaceous, in New Zealand become shrubs, often of considerable size. Of these, Veronica is especially notable, and the Compositae, like Senecio and Olearia (near Aster), are ordinarily shrubby species. Herbaceous plants are rather in the minority, but several familiar-looking buttercups, geraniums, and oxalis were noted, and a couple of inconspicuous orchids, one of which, *Thelymitra* sp., was much less beautiful than some of the Australian species.

A coarse sedge, *Mariscus ustulatus*, and two species of Astelia, *A. Banksii* and *A. Cunninghamii*, were abundant, and, on rocks near the shore, *Salicornia australis*.

[3] Cockayne. New Zealand plants and their story, p. 41. Wellington, 1919.

As elsewhere in New Zealand, ferns were abundant, and in addition to the ubiquitous *Pteridium aquilinum*, several species of Polypodium, Aspidium, Asplenium, and Pellaea were seen.

A number of small epiphytic hepatics were common, and in one place an abundant growth of the familiar *Lunularia cruciata*, which apparently is native in both Australia and New Zealand.

Wellington

The only other region in the North Island visited by the writer was Wellington, whose fine harbor, surrounded by rugged mountains, opens into Cook's Strait between the two main islands of New Zealand. Back of the harbor rise very steep hills, which at the time of my visit were ablaze with golden broom, extensively naturalized in many parts of the country.

There is a pretty botanical garden in Wellington, which was gay with spring flowers. I was struck by the luxuriant growth of some Californian conifers, especially the very tall and symmetrical Monterey pines, a tree which is extensively planted for timber in New Zealand. To the botanist the most interesting feature of the garden is a small ravine which harbors a remnant of the forest which originally clothed much of the country about Wellington. Here one may see in their natural environment many of the characteristic trees, shrubs, and herbaceous flowering plants, as well as a good many ferns, liverworts, and mosses of the region.

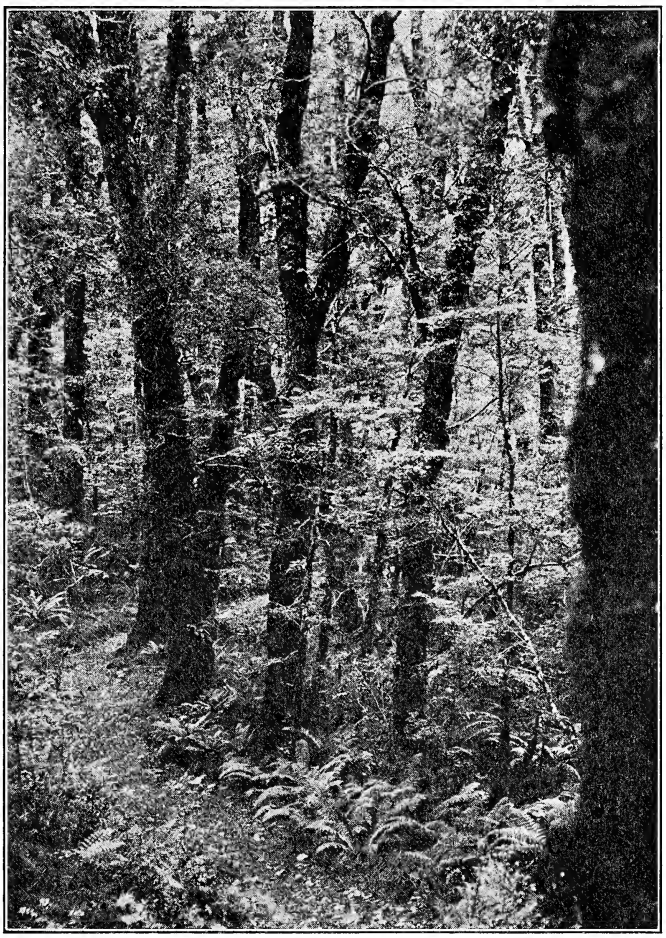
I made a visit to this interesting spot in company with Professor T. Kirk of the Wellington University, and to him I am indebted for an introduction to many of the characteristic local plants. Ferns were abundant, including fine specimens of the magnificent tree-fern *Cyathea medullaris*, as well as á number of small Hymenophyllaceae, species of Blechnum, Polypodium, and Asplenium. Some unusually interesting liverworts were noted, both thallose and leafy species, and were a foretaste of the rich harvest of these interesting plants afterwards collected in the rain-forest of the South Island.

The trees and shrubs were much the same as those in the Auckland district. Two common shrubs, *Pittosporum tenuifolium* and *P. crassifolium*, were familiar, as they are common in California gardens, and *Sophora tetraptera*, one of the few showy-flowered New Zealand shrubs, is also occasionally seen in cultivation.

The Myrtaceae, so abundant in Australia, are much less in evidence in New Zealand, with the exception of the two common Leptospermums and Metrosideros. The commonest species, aside from the latter, are *Myrtus bullata* and *Eugenia maire*.

The Proteaceae are even more poorly represented than the Myrtaceae, there being only two species in New Zealand, *Persoonia Toru* and *Knightia excelsa*. The latter is a handsome tree with foliage somewhat like that of the Australian Banksias. Besides the New Zealand species, there are two others in New Caledonia.

Other characteristic shrubs and small trees were several shrubby Compositae (Senecio, Olearia), Melicytus, Elaeocarpus, Pennantia, Panax, and several others. To me the most interesting tree was *Fuchsia excorticata*, one of three New Zealand species of this characteristically South American and Mexican genus. It is a small tree, with smooth, reddish bark and



TEXT FIG. 3. Beech-forest interior. The fern is *Polystichum vestitum*. Photographed by Mr. W. D. Reid.

typical Fuchsia leaves and flowers, so that one recognized it at a glance. *Podocarpus dacrydioides* and *Dacrydium cupressinum* are quite common, and in the low ground is an interesting tree, *Laurelia Novae Zealandeae*, which has highly developed buttress roots functioning as pneumatophores.

Across the harbor from Wellington is a forest very different from the rain-forest in the immediate vicinity of the town. This is an almost pure stand of two species of beech, *Nothofagus fusca* and *N. Solanderi*. The

forest is more open than the rain-forest, and the trees quite suggest the northern beeches in habit, but the evergreen leaves are much smaller. They also recall the alders of the Pacific Coast. In the South Island the beech forests are much more extensive and widespread.

In the beech forest at Day's Bay near Wellington, there was a moderate undergrowth of shrubs and young trees. Among the shrubs are two species of Cyathodes (*Epacridaceae*), *Panax arboreum*, Geniostoma, and Coprosma. *Gaultheria antipoda*, a common low shrub, and a pretty Clematis, *C. Colensoi*, recalled their American relations.

Cook's Strait, separating the two main islands, does not, apparently, act as a barrier to plant migration, there being little difference in the vegetation on the two sides of the strait. This would indicate that the separation of the islands occurred at a date too recent to result in any appreciable change in the vegetation.

The South Island

The South Island, owing both to its higher latitude and to the presence of the lofty chain of the Southern Alps, shows a greater diversity of climate than is the case in the North Island. The snow-clad Alps intercept a large part of the moisture from the westerly winds, and the result is seen in the treeless Canterbury plain east of the range, and the still dryer region of Central Otago in the south. On the other hand, the strip of country, Westland, between the west coast and the mountains, has the heaviest rainfall in New Zealand, some stations registering 200 inches annually. This wet district supports a magnificent forest of truly tropical luxuriance.

The important city of Christchurch is surrounded by the Canterbury Plain, an open grassland somewhat suggestive of our prairies, and like them offering exceptionally favorable conditions for agriculture, and now the most important agricultural district of New Zealand. The region, naturally, is mainly covered with tussock grasses, and there is very little forest growth.

Through the kindness of Dr. Charles Chilton of Canterbury College, and Dr. L. Cockayne of Wellington, I was shown the most interesting botanical features of the neighborhood.

There is a small patch of forest, the "Riccarton Bush," in Christchurch, growing in low ground and probably the last survivor of other similar groves, perhaps comparable to the "oak openings" of our own eastern prairies. The other exception to the prevailing grass formation is the rugged Bank's Peninsula south of Christchurch, where there is still a considerable forest growth in the sheltered places.

As might be expected from its position, the climate of the Canterbury plain is less equable than that of the west coast, the winter being marked by sharp frost and the summer heat being much greater. The extremes are still greater in Central Otago.

Christchurch has a very attractive botanical garden, which was in the

full flush of its late spring floral display. The great charm of the garden at this time was the luxuriance and freshness of the young foliage and the masses of Rhododendrons, lilacs, and other familiar shrubs in full bloom. A very beautiful collection of Leptospermums was noteworthy. These ranged in color from pure white, through every shade of pink and red to the deep crimson of *L. Nichollsii*, which is supposed to be a mutant from the white *L. scoparium*. The herbaceous borders were gay with a great variety of hardy perennials which are very successfully grown in New Zealand. Indeed, one of the most attractive features both of New Zealand and of Australia is the general cultivation of flowers and the beauty of the gardens everywhere.

TEXT FIG. 4. Tussock-grass formation, South Island. *Poa caespitosa*. Photographed by Mr. W. D. Reid.

The most extensive and interesting trip was to the west coast over the mountains by way of the Otira Gorge, one of the finest sights in New Zealand. On this expedition I was fortunate in being accompanied by Dr. L. Cockayne, whose numerous and important contributions to the botany of New Zealand are well known to all students of the flora of the country. Under his able guidance I was introduced to the most interesting of all the New Zealand forests, and my brief stay in Westland was a very

busy and delightful one. I am also greatly indebted to Dr. Cockayne for a large number of photographs, some of which are included in the present article.

The journey across from Christchurch was intensely interesting. The Canterbury plain and the lower slopes of the mountains adjoining were barren-looking, with little vegetation except tussocks of coarse grass, the principal species being *Festuca Novae Zealandeae* and *Poa caespitosa*, the former predominating.

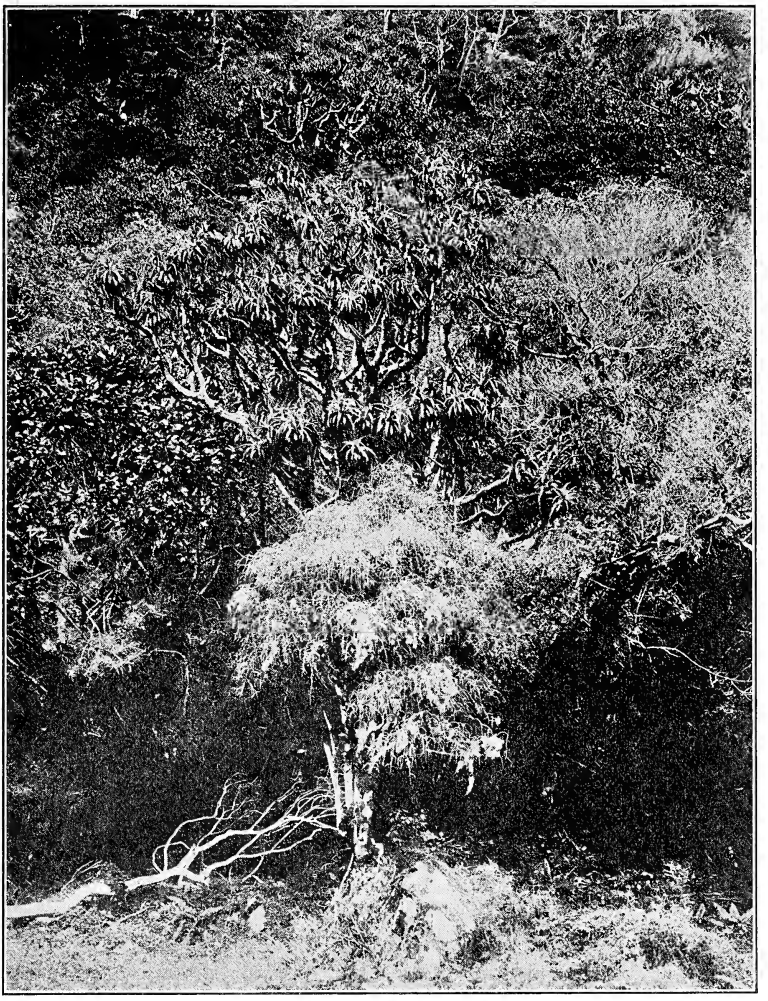
As the train proceeded, there was a sudden change in the vegetation, marking the beginning of the rainy western district. The bare grassland suddenly stopped and the train entered a region of heavily wooded mountains, the forest being composed almost exclusively of the mountain beech, *Nothofagus Cliffortioides*. This forest is very dense, and there is little undergrowth, but the trees are much lower than those seen in the beech forest near Wellington. In exposed situations the mountain beech may be reduced to the dimensions of a shrub.

Arthur's Pass, the summit of the road, is of about 3000 feet elevation, and from this point the increasing moisture on the west side of the mountains becomes more and more evident. The country around the pass is an open, more or less rocky moorland, with no large trees, but with extensive thickets of shrubs and stunted trees along the roadside and in the more sheltered places. Two conifers, *Libocedrus Bidwillii* and *Phyllocladus alpinus*, belong to this sub-alpine region, but only the latter was seen by me, a shrub with leaf-like cladodes like those of the species seen in the North Island.

The sub-alpine scrub is composed of many species, among which shrubby Compositae play an important rôle. Among these Olearia, Senecio, and Traversia take first place. The genus Olearia is highly developed in New Zealand; it comprises a number of species with showy flowers, and *O. ilicifolia* has handsome glossy, holly-like leaves. Several species of Veronica are also common, and vary a good deal in appearance, some having rather conspicuous thick leaves, others small, closely imbricated ones, like a cypress. Many of the hundred New Zealand species have racemes of showy blue or purple flowers, and are common in cultivation.

Much the most striking member of the sub-alpine scrub is a small tree, strongly resembling a Yucca or Dracaena, but in reality belonging to the Epacridaceae. This is *Dracophyllum Traversii*, very common along the roadside and certain to attract the attention of the most casual traveler. The narrow Yucca-like leaves are reddish in color and at once catch the eye. The genus is exclusively Australasian, except for certain species in New Caledonia.

Other characteristic plants of the scrub were a leafless leguminous shrub, *Carmichaelia* sp., and *Pseudopanax lineare*. On banks along the roadside the mountain flax, *Phormium Colensoi*, was abundant, a smaller plant than *P. tenax*.



TEXT FIG. 5. Sub-alpine scrub, Arthur's Pass. Center, *Dracophyllum Traversii;* in front, *Suttonia divaricata*. Photographed by Dr. L. Cockayne.

Some of the herbaceous plants of the open stony "fell-field" are very striking. Easily first in point of beauty is the magnificent *Ranunculus Lyallii*, the finest of all buttercups. This noble plant is very abundant about Arthur's Pass and was in full bloom at the time of our visit. Unfortunately, boys had raided the more accessible places and stood with armfuls of the flowers which they offered for sale, so that few were left near the road. This is the most beautiful of all New Zealand flowers. The great peltate entire leaves, sometimes 8 or 10 inches in diameter, are borne on long petioles, and the snow-white flowers, two inches or more in diameter, are in clusters of 25 or more and form a beautiful bouquet above the rich glossy foliage.

Several species of Celmisia (Compositae) are abundant, and some have very handsome daisy-like flowers, and other Compositae, Senecio and Chrysobactron, contribute to the floral display; but next to the Ranunculus the most attractive flower was *Ourisia macrocarpa*, a low-growing plant allied to Mimulus, with abundant white flowers. Ourisia is a genus which New Zealand and Tasmania share with sub-antarctic South America.

The composite genus Raoulia, best known by the curious sub-alpine "vegetable sheep," *R. eximia*, has a number of common and widespread species, forming dense cushions on rocks and in the dried-up beds of streams, and in similar localities.

Another peculiar and abundant sub-alpine plant is *Aciphylla Colensoi*, an umbellifer, but with its stiff, dagger-like leaves looking much more like a dwarf Yucca.

The coach drive through the Otira Gorge, descending to the west from Arthur's Pass, is one to be long remembered. The lofty walls of this magnificent canyon are extremely steep, and clothed from crest to base with a dense jungle of evergreen trees and shrubs. The great luxuriance of the vegetation testifies to the heavy precipitation, and the New Zealand rain-forest reaches its culmination in the excessively wet Westland province.

The increasing moisture of the western slope is evidenced as one descends by the greater profusion of ferns, liverworts, and other herbaceous moisture-loving plants like violets, Hydrocotyle, Nertera, and similar things. Particularly interesting was an abundance of *Gunnera albocarpa*, a genus especially developed in New Zealand. The roadside banks were richly clothed with these and many other interesting plants. As we descended, tree-ferns, absent from the higher elevations, appeared and became more abundant as the coast was approached.

Several days were spent on the west coast, which proved most interesting. In this region one can still see untouched forest of the most luxuriant character. In these rain-forests ferns and liverworts abound, and the latter attain a size and luxuriance that I have never seen equaled, even in the tropics.

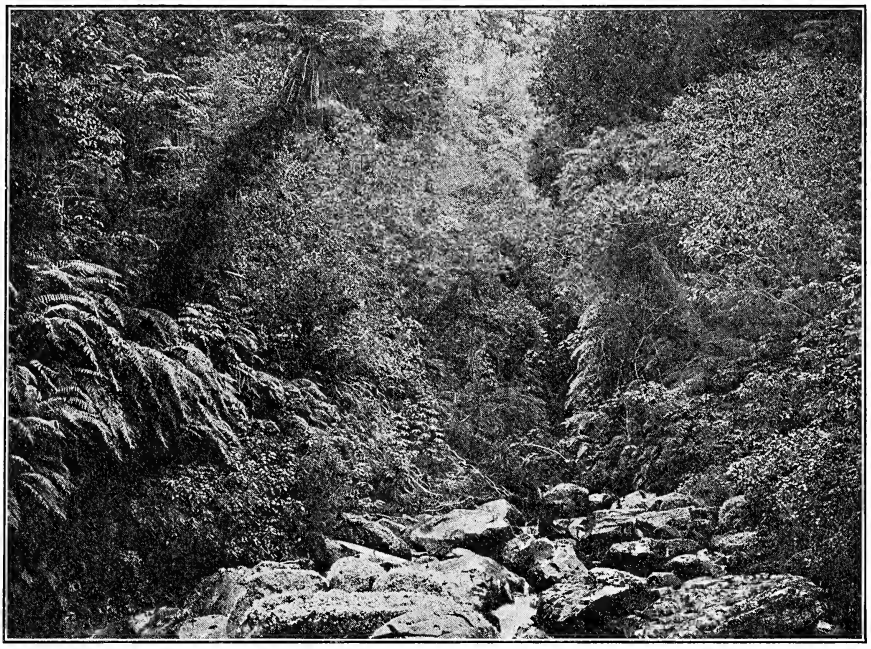
The Westland rain-forest is dominated by two genera of Taxads, Podocarpus and Dacrydium, and Cockayne[4] designates this type of rain-forest "Taxad forest."

Where the land is low and swampy, *Podocarpus dacrydioides* forms almost pure stands. It is known locally as "white pine," and, as its timber is valuable, the forests are being rapidly cut down. These forests are very characteristic in appearance. The trees grow close together, and their very tall, rather slender trunks are bare for most of their height, with relatively small crowns of foliage. There is comparatively little undergrowth, and lianas and epiphytes are much less developed than in the typical rain-forest, so that the crowded bare, mast-like trunks present a

[4] Vegetation of New Zealand, p. 133.

very different picture from the mixed rain-forest of the same district, with its trees loaded with epiphytes, the trunks sometimes completely hidden by the dense masses of creepers and epiphytic growths.

While the undergrowth in the white-pine forest, especially where there is much standing water, is scanty, there are all degrees between this condition and the heavy undergrowth of the mixed Taxad rain-forest.



TEXT FIG. 6. Westland rain-forest. Photographed by Dr. L. Cockayne.

This mixed Taxad forest of Westland is one of extraordinary luxuriance. The vegetation is entirely evergreen in character, and the profusion of lianas and epiphytes, and the magnificent development of tree-ferns, give the impression of a true tropical jungle. This impression is not lessened when the character of the vegetation is more closely analyzed; for a large proportion of the trees and shrubs are related to tropical types, rather than to those of more temperate latitudes. To the northern botanist it comes as a genuine surprise to see a forest of this character in latitudes corresponding, in the United States, to that of Buffalo or Milwaukee.

The two most important trees are *Podocarpus dacrydioides*, already mentioned, and *Dacrydium cupressinum*, a beautiful tree with drooping cypress-like branches.

While there are no palms, like the Nikau of the North Island, their place is taken by the magnificent tree-ferns, which are everywhere abundant and often 30–40 feet high. The most common is *Dicksonia squarrosa*, a much

more beautiful species than the more familiar *D. antarctica*. *Hemitelia Smithii*, a smaller, but handsome species, also occurs, but is more abundant farther south.

Perhaps nothing adds more to the tropical aspect of the forest than the giant lianas. Two of these, *Rhipopogon scandens*, a liliaceous climber with long, bare cable-like stems looping from tree to tree, and the root-climber, *Freycinetia Banksii*, are especially noteworthy. The screw-pine family, to which the latter belongs, is essentially a tropical one, and largely developed in the Malayan region.

Of the trees, a second species of Podocarpus, *P. ferrugineus*, and the widespread *Weinmannia sylvicola* may be mentioned, as well as a number of smaller trees and shrubs. Among the commonest of these are *Quintinia acutifolia*, *Ascarina lucida* (Chloranthaceae), a striking shrub with black twigs and shining green leaves; *Aristotelia racemosa*, *Pseudopanax crassifolia*, *Coprosma foetidissima*, *Elaeocarpus Hookerianus*, and several species of Metrosideros, including *M. florida*, a climbing species.

In this wet forest many ferns find a congenial home and are extremely abundant. In addition to the more familiar northern genera, there are a number of peculiar types of which the most striking is the genus Leptopteris, of the Osmundaceae and sometimes united with Todea. There are two New Zealand species, *L. superba* and *L. hymenophylloides*. The finely cut, plumy leaves have the delicate translucent texture of the true filmy ferns. The latter are very abundant and reach a wonderful development. The species of Hymenophyllum are more numerous than Trichomanes, although there are several species of the latter, including the common *T. reniforme* already referred to. This striking species is very common, its entire kidney-shaped leaves of a vivid green and thicker than those of most Hymenophyllaceae. The numerous species of Hymenophyllum grow upon the ground and on fallen logs and stumps; but they are most abundant upon the trunks and branches of trees. The stems of tree-ferns are a favorite habitat for these beautiful ferns, as well as for other epiphytic growths.

Epiphytic species of Lycopodium and the curious Tmesipteris are features of this rain-forest, and there is a wealth of mosses and liverworts, as well as many very large and conspicuous lichens. Of the epiphytic mosses a species with long pendent branches (*Weymouthia Billardieri*) was especially notable.

This rain-forest is extremely rich in mosses and liverworts, and includes some of the most remarkable members of these groups. Of the mosses, the giant *Dawsonia superba* and the liverwort-like *Cyathophorum pennatum* may be mentioned. The liverworts are remarkable for both their great abundance and their size. They form immense cushions and thick carpets on the floor of the forest, and among them are several of the giants of the class. Of the foliose type, several enormous species of Plagiochila, Tri-

chocolea, and Gottschea are extremely common; and of the thallose species, the gigantic *Monoclea Forsteri* formed great mats in wet places.

Of the less conspicuous but interesting thallose liverworts may be mentioned species of Hymenophyton, Symphyogyna, and Pallavicinia, and at one place I was fortunate enough to find the rare *Treubia insignis* (?) and a species of Calobryum.

Of the common herbaceous plants, one of the most interesting is a trailing plant, *Nertera depressa*, belonging to the Rubiaceae. This species is common to temperate South America and Australasia.

On my return to Wellington I had an opportunity, in Nelson, of meeting Dr. J. E. Holloway, whose studies on the development of the New Zealand Lycopodiums and Tmesipteris are of great importance. I am greatly indebted to Dr. Holloway for valuable material of these interesting plants.

I had no opportunity of visiting the outlying islands of New Zealand. An excellent general account of these is given by Cockayne in his book, "New Zealand plants and their story," already referred to. As might be expected, the floras of these islands in the main are composed of species common to the main islands, but all the smaller islands show a certain number of endemic species.

The Kermadec islands to the north have several species of tropical affinities which do not reach the North Island. These include a second species of palm, *Rhopalostylis Cheesemannii*, and two tree-ferns, one of which, *Cyathea Kermadecensis*, is reported to reach a height of 70 feet.[5]

The Chatham Islands to the east have a total of 236 species, including two endemic genera and 31 endemic species.

To the south lie the sub-antarctic islands extending to 55° south latitude. These islands have an extremely harsh climate but not excessively cold, apparently much like that of Tierra del Fuego and southern Patagonia. The rainfall is heavy, and they support a surprisingly rich flora of ferns, mosses, and liverworts, as well as a low forest in the most favorable situations in the Auckland Islands, in latitude 50°.

The predominant tree of this forest is *Metrosideros lucida*, which is associated with species of Panax, Suttonia, and Coprosma, all genera of Malayan affinity—a surprising southward extension of these sub-tropical types. A tree-fern, *Hemitelia Smithii*, has also been reported from this region.

There is a rich herbaceous vegetation with some very showy flowers, including species of gentians, buttercups, and others. The vascular plants of the sub-antarctic islands number 188 species, of which 53 are endemic, each island having some peculiar species.[6]

Reference has already been made to the abundance of lianas and epiphytes in the New Zealand rain-forest. The former are of several different

[5] Oliver, R. B. The vegetation of the Kermadec Islands. Trans. N. Z. Inst. 42: 118. 1910.

[6] Cockayne, L. New Zealand plants, pp. 129–143.

types. Some, like *Fuchsia Colensoi* and species of Rubus, are "scramblers"; others are twiners or tendril climbers, and others, like species of Metrosideros and Freycinetia, are root climbers. The species of Rubus are noteworthy, especially the common *R. Australis*, which develops huge cable-like stems, clambering to the tops of the tallest trees. This giant bramble is a novel feature to a northern botanist, and the thorny tangles are almost as bad as the tropical rattans. A number of ferns may be included in the list of climbers. In addition to *Lygodium articulatum*, whose twining leaf-stalks are said to reach a length of 100 feet,[7] there are a number of species which may be described as root climbers. The most striking of these is *Blechnum filiforme*, which begins life on the floor of the forest and later climbs the trunks of trees. There is a marked difference in the leaves of the two stages, those of the climbing plant being much larger. Other common climbers are *Rhipopogon scandens* (Liliaceae), *Muehlenbeckia* spp., Parsonsia (Apocynaceae), a twiner with pretty white sweet-scented flowers. Several species of Clematis are the commonest tendril climbers.

Epiphytes

Like the climbing plants, the epiphytic vegetation shows much variety. Some species are "temporary" epiphytes, like *Metrosideros robusta*, which begins life as an epiphyte but finally becomes rooted in the ground. Species of Dracophyllum and Griselinea behave in much the same way.

The "permanent" epiphytes comprise a great variety of bryophytes, lichens, and pteridophytes. The most conspicuous epiphytic monocotyledons are species of Astelia, but these are not exclusively epiphytic. The epiphytic orchids, as already indicated, are inconspicuous species, but may belong to such tropical genera as Dendrobium, Bulbophyllum, and Earina. Of the dicotyledons, Pittosporum includes two epiphytic species, and *Griselinea lucida*, a very conspicuous shrub, is frequently, but not always, an epiphyte.

The Principal Elements in the New Zealand Flora

In considering the New Zealand flora as a whole, one is at once impressed by the predominance of Malayan elements in the vegetation, especially as regards trees and shrubs.

It is very evident that the most important features of the flora have been derived from the tropical regions to the north, and, although the climate of New Zealand is a decidedly temperate one, many of these tropical immigrants have adapted themselves to the much lower—but even—temperatures of the South Island.

Of course the tropical element is more pronounced in the North Island, and a good many species are confined to it; but many unmistakably tropical

[7] Hooker, J. D. Handbook of the New Zealand flora, p. 385. 1867.

genera, like Metrosideros and Freycinetia, are found practically throughout the whole of New Zealand.

It is probable that the gymnosperms, Agathis, Podocarpus, Dacrydium, are of northern origin, as they are all widespread throughout the Malayan region. Metrosideros, Eugenia, Beilschmiedia, Sideroxylon, are examples of trees of Malayan affinities, and the same is true of a number of the characteristic trees and shrubs. The Nikau palm, Cordyline, and Freycinetia, the latter belonging to the almost exclusively tropical family Pandanaceae, are certainly derived also from the north. The prevalence of these and other similar tropical genera explains the markedly tropical aspect of the New Zealand rain-forest, even in the colder parts of the South Island.

The fern vegetation also has much in common with the Indo-Malayan and Polynesian regions.

The Australian Element

The relations of the New Zealand and Australian floras are somewhat complicated. A good many common genera, like Freycinetia, Agathis, Podocarpus, Dendrobium, and most of the ferns, are widespread, and do not necessarily imply direct connection between the two countries; but on the other hand the occurrence of genera, or even species, confined to Australia and New Zealand makes it certain that they must have migrated from one country to the other; but in some cases it seems quite as likely that New Zealand, and not Australia, was the place of origin.

35 genera (or sub-genera) are confined to New Zealand and Australia.[8] Some of these are much better developed in Australia than in New Zealand, and are presumably of Australian origin. Such are the orchids Caladenia and Thelymitra; Epacris, Swainsonia, and Persoonia. Some of the common genera have a single species common to the two countries, *e.g.*, the grass *Echinopogon ovatus* and the liliaceous *Herpolirion Novae Zealandeae*. Other genera have two species, one each in Australia and New Zealand, *e.g.*, Ackama, a small tree related to Weinmannia. An interesting case is that of Clianthus. The gorgeous "Sturt pea" (*C. Dampieri*) of the deserts of South and West Australia, has its counterpart in rainy Westland in the beautiful *C. puniceus*. The latter case is certainly a very puzzling one.

A New Zealand origin seems probable for the following: Gunnera (sub-genus Milligania), 10 New Zealand, 1 Australia; Celmisia, 55 New Zealand, 1 Australia; Aciphylla, 25 New Zealand, 1 Australia. Several other genera are in the same category.

It is significant that many distinctively Australian types are quite absent from New Zealand, and others are very poorly represented. The two leading Australian genera, Acacia and Eucalyptus, are unknown to New Zealand, and the Proteaceae, with nearly 700 Australian species, have but two in New Zealand. The Myrtaceae of New Zealand comprise barely twenty

[8] Cockayne, L. New Zealand plants, p. 206.

species, compared with over 800 in Australia. In short, the differences between the floras of the two main divisions of Australasia far outweigh the resemblances, and we must conclude that each country owes comparatively little to the other for contributions to its flora.

The Fuegian Element

Of special interest is the occurrence in New Zealand of many species either identical with, or closely related to, those of Patagonia and the adjacent regions.

Between the Chatham Islands east of New Zealand, and Juan Fernandez off the coast of Chile, lies almost exactly one fourth the circumference of the globe, and there is absolutely no intervening land. In spite of this, the relationships between the vegetation of the two regions are perfectly obvious, and indicate unquestionably the former existence of land communication of some sort. No less than 68 genera are common to the two regions, and many of these are peculiar to them, although there are widespread genera as well.

Scottsberg,[9] one of the more recent students of this interesting problem, recognizes three categories of these common genera: 1, predominant Australasian genera, with American representatives; 2, Andine or Patagonian genera, with Australasian representatives; and 3, bicentric genera, considered to be remnants of an ancient antarctic flora. Examples of the first category are Astelia, Lomatia, Orites, Drimys, Drapetes, Pseudopanax. The second type may be represented by Enargia, Fuchsia, Pernettya, Jovellana, Ourisia. Of the third, Oreobolus, Libertia, Nothofagus, Laurelia, Muehlenbeckia, Gunnera may be mentioned.

Twenty-five species are recognized as identical between New Zealand and Patagonia, and about the same number are doubtfully distinct.

As we have already indicated in a previous paper, this Fuegian element is also strongly developed in Tasmania, and to a lesser degree in Victoria and the higher mountains of New South Wales and Queensland.

Conclusion

It is generally admitted that the present land surface of New Zealand is very much reduced from an earlier period. This is indicated both by the extent of shallow sea surrounding the islands, and also by the distribution of the vegetation. Both of these facts point to a union of the islands into a single mass of much greater extent, and this at a recent period, geologically.

The great preponderance of the Malayan element in the vegetation indicates land extensions to the north, and this is borne out by soundings. There are two submarine ridges extending northward from New Zealand.

[9] Scottsberg, C. Notes on the relation between the floras of subantarctic America and New Zealand. Plant World **18**: 129–142. 1915.

One of these, the Kermadec ridge, extends as far north as the Samoan group, while the other trends toward New Caledonia and tropical Australia. Lord Howe Island and Norfolk Island are connected with this ridge, and their flora reveals unmistakable relationships to that of New Zealand.[10]

Whether such truly Australian types as the Epacridaceae and Candolleaceae reached New Zealand *via* this northern route, or whether they came from the south, we have no means of determining at present.

How the conspicuous Fuegian flora reached Australasia has been the subject of much discussion, and there is difference of opinion on this subject. To the writer it seems inconceivable that the majority of the many common types could have been transported except by means of more or less continuous land connections. This conclusion is emphasized by the numerous correspondences in the fauna, especially such forms as fresh-water fishes and Crustacea, which could hardly have migrated across several thousand miles of open ocean.[11]

The simplest explanation of the correspondence in both the fauna and flora of these widely separated regions would be the assumption of a former northward extension of the present Antarctic continent, or perhaps the existence of an extensive archipelago occupying this region. This land might be supposed to have developed a more or less uniform flora, such as existed in similar latitudes of the northern hemisphere in the late Tertiary. The subantarctic genera, like Nothofagus, Laurelia, Gunnera, etc., may be the remnants of this ancient antarctic flora which have survived in their present widely sundered habitats. Possibly also the ancestors of other types now confined to Australia or New Zealand may have had a similar origin.

This view is strengthened by the discovery of plant fossils in the existing antarctic regions. Especially interesting was the discovery by a Swedish expedition on Seymour Island, near the Antarctic Continent, of fossils closely related to living New Zealand species. Among these were remains of a Knightia, apparently very similar to *K. excelsa*—one of the two living New Zealand Proteaceae.

A migration of these ancient antarctic plants into South America, Australasia, and South Africa, and the subsequent complete isolation of the respective regions, would explain many of the apparent anomalies in the distribution of the existing subantarctic floras, as well as the source of the ancestors of the peculiar floras of Australia and South Africa.[12]

The student of the New Zealand flora is at once impressed by the extension of so many tropical and subtropical genera far beyond the latitudes

[10] Oliver, W. R. B. The vegetation and flora of Lord Howe Island. Trans. N. Z. Inst. 49: 24–161. 1915.

[11] Chilton, C. The subantarctic islands of New Zealand. 2 vols. Wellington, 1909.

[12] Dr. J. C. Willis has published a series of papers (Annals of Botany, 1916–1920) in which he discusses in great detail the flora of New Zealand and the sources from which it has been derived. His conclusions agree closely with the views expressed in the present paper.

in which we should look for them. It is evident that their existence does not depend upon very high temperatures, but in the absence of extreme cold, and provided with copious and constant moisture, many species of tropical affinities may flourish in high latitudes. This fact has an important bearing on the question of changes in climate in earlier geological time.

It would not seem necessary to assume any radical changes in the earth's climate as a whole to explain the remains of warm-temperate types in the far north, where now conditions are impossible for their existence. Could we replace the existing continental conditions by an archipelago bathed by warm ocean currents, there is no reason why, in the northern hemisphere as in the southern, the plants of the lower latitudes should not extend their range toward the pole, far beyond their present limits.

In conclusion, the writer would like to thank his botanical colleagues in New Zealand for the assistance of many kinds given him in his travels through the country; but he would also express his great appreciation of the kindness of various other gentlemen, official and otherwise, who helped to make his visit a very successful one. He feels under special obligation to Mr. J. D. Gray of Wellington, Secretary of the Department of Foreign Affairs.

STANFORD UNIVERSITY

EXPLANATION OF PLATE XXXVII

Rain-forest, North Island. Nikau palm and tree-ferns.

CAMPBELL: AUSTRALASIAN BOTANICAL NOTES.

HYDROGEN-ION CONCENTRATION AS RELATED TO THE FUSARIUM WILT OF TOMATO SEEDLINGS[1]

EVERETT CLIFTON SHERWOOD

(Received for publication January 11, 1923)

It has recently been shown by Jones (15) that phytopathologists should give especial consideration to the relations of environment to predisposition. Johnson and Hartman (14) have emphasized particularly the importance of certain soil factors in the development of those diseases which attack the underground parts of plants. The investigations reported in this paper were planned to determine the possible relation of soil reaction to the development of the wilt of tomato (*Lycopersicon esculentum* Mill.) caused by the soil parasite *Fusarium lycopersici* Sacc. The work was coincident with Clayton's recently published studies on the influence of temperature (3) and of moisture (4) upon the development of this disease and was designed to supplement those investigations. It was, indeed, begun in immediate association with Dr. Clayton, and the author is indebted to him both for the initial cultures of Fusarium used for soil inoculation and for much critical attention in the consideration of evidence as the problem was developed.

The only paper coming to the attention of the writer which deals directly with the influence of soil reaction upon the development of the Fusarium wilt of tomatoes is that of Edgerton (5). He reports that an application of lime at the rate of ten tons per acre reduced the disease in the seed bed from 51.0 percent to 4.4 percent. In the field the wilt was reduced in the Acme variety from about 77 percent to 40 percent, and in the Earliana from about 75 percent to 23 percent. The reaction of the soil before liming was, however, not reported. He found also that a variety of tomatoes showing high susceptibility to the disease in the seed bed manifests the same degree of susceptibility in the field.

Few references were found with respect to the effect of liming on the growth of tomatoes. Mooers (17) concluded that they are only moderately benefited. He based his conclusions on tests made in soils where liming was necessary to secure a fair crop of clover. Hartwell and Damon (9) found that liming resulted in a slight decrease in yield on one plot which required 2500 pounds of calcium oxide per acre to neutralize the soil according to the Vietch lime-requirement method. They reached the conclusion that tomatoes are tolerant of a moderate degree of acidity. The exact reaction of the soil after liming, however, was not determined.

[1] Investigations carried on at the University of Wisconsin under advisory relations with Professor L. R. Jones, to whom the author is indebted for counsel and criticism.

Hoagland and Sharp (13) explain that

> The criteria heretofore used for judging the reaction of soils do not always permit of an accurate distinction between soils of different reactions. Some soils may be judged as acid from the standpoint of certain lime requirement methods, when in reality their reaction may be alkaline.

Hoagland (11) calls attention to the common assumption that most agricultural plants require a slightly alkaline reaction in the soil, and cites the fact that good crops of barley, oats, beans, potatoes, onions, corn, and asparagus were grown on a California peat soil having an acid reaction ranging from pH 4.5 to 5.4. In another paper Hoagland (12) reports experiments with the growing of barley seedlings in partial nutrient solutions of like osmotic pressure but having a considerable range of hydrogen- and hydroxyl-ion concentrations. For similar divergences from the neutral point, the hydroxyl ion was found to be more toxic than the hydrogen ion. A reaction of approximately pH 8.3 was found to be distinctly injurious, while a reaction of pH 5.0 produced no injury.

Little information is available concerning the behavior of fungi in general toward varying degrees of hydrogen- and hydroxyl-ion concentration. Webb (22) has recently reviewed the literature on the toxicity of acids and alkalies to the growth of fungi. Most of the information given in the literature is stated in such terms as "alkaline," "slightly acid," "strongly acid," or as some percentage of acid or base added. Webb (22) experimented with spore gemination in culture solutions adjusted to various hydrogen-ion concentrations. He found that increasing concentrations of hydrogen ions from approximately neutral to pH 3.1 influenced favorably the germination of spores of all the forms studied. Germination was not inhibited until a concentration of pH 2.8 was reached. Spores of a species of Fusarium were found capable of germinating over the entire range from pH 2.8 to 10.0.

Meacham (16) reports that the growth of four wood-destroying fungi, cultured in synthetic and malt-extract media, was not inhibited until approximately pH 3.0, and that the limiting value was approximately pH 1.7. Hawkins and Harvey (10) tested the growth of *Pythium debaryanum* potato-juice cultures adjusted to known hydrogen-ion concentrations with sodium phosphate buffer mixture. The fungus grew well in all cultures ranging from pH 3.4 to 6.8. Growth was poor in the cultures adjusted to pH 3.1 and in those below the neutral point testing pH 7.3 and 8.4.

In his study of the growth of the common potato-scab organism at various hydrogen-ion concentrations, Gillespie (7) found that the growth at pH 5.2 was slower and generally less vigorous than at higher concentrations. While some of the strains tested grew at pH 5.2, the growth in these cases was accompanied with a marked decrease in acidity. Gillespie and Hurst (8) report the examination of a considerable number of soils from northern Maine and show that a very close correlation exists between the hydrogen-

ion concentration and the occurrence of the common potato scab. A similar correlation was found to exist in certain soils of different origin and types. Soils found to have hydrogen-ion concentrations of pH 5.2 or lower rarely produce scabby potatoes. The conclusion reached was that "the limiting zone of the hydrogen ion exponent for the potato scab organism appears to be about the same for the soil as had been previously found for culture media".

General Considerations

Besides the purely environmental conditions which may influence the parasitism of the tomato plant and the development of the disease, there must be taken into account the degree of susceptibility of the host and the virulence of the causal organism. Clayton did some preliminary work (3) with three strains of *Fusarium lycopersici* isolated from tomatoes which came respectively from Louisiana, Tennessee, and Indiana. He found considerable differences in the relative pathogenicity of the strains; these differences seemed stable and persistent. Such differences in pathogenicity among different strains of *Fusarium lycopersici* have been reported by Edgerton (5). In this connection he concludes that the virulence of the organism is not diminished to any appreciable extent when the organism is grown on artificial culture media for a period of two years or even more. The writer's experience accords with that of Clayton and Edgerton, namely, that, within reasonable limits at least, the age of the culture does not introduce a factor of appreciable variability.

Of the various conditions of soil environment which may influence experimental results, Clayton (3) concluded that temperature is the most important single factor. His results show that soil temperature of about 28° C. is most favorable for the development of the disease. He also determined that the optimum soil-moisture condition occurred at about two thirds of its moisture-holding capacity.

It was assumed that whatever effect soil reaction might have on the wilt would be shown most conclusively under conditions otherwise favorable for the development of the disease. An attempt was made, therefore, to keep the temperature of the greenhouse at 28° C., though fluctuations occurred, as will be noted in connection with each experiment. The moisture content of the soil was not accurately controlled, but water was so applied as to keep the plants in good growing condition, and Clayton's correlated experiments showed that this approximated two thirds of the moisture-holding capacity of the soil.

The experiments were conducted with two types of soil, a sandy loam and a silt loam, obtained from fields in southern Wisconsin. Both were naturally acid soils which had been previously found by the Department of Soils of the University of Wisconsin to give a strongly acid reaction when tested by the Truog (20) method. With these as a base, various adjustments of

the reaction were made, principally by the use of calcium carbonate and calcium oxide. The treatment of the soils was similar in all the experiments with the exception of the addition of the materials necessary to secure the desired reaction. Disregarding slight fluctuations in moisture and temperature relations, the conditions for all the experiments were similar.

Soil Reaction and its Determination

It is well known that most of our common agricultural plants thrive best in soils well supplied with calcium carbonate. It has been very generally assumed, therefore, that many plants require an alkaline reaction in the soil. Measurements of soil acidity have been designed for the purpose of determining the amount of lime required in order to bring about an alkaline condition, or to adjust the reaction to some arbitrarily selected end-point. A sufficiently accurate method of determining the end-point reached after the attempted adjustment has been lacking. Within the past few years, however, the hydrogen electrode has come to be used as an indicator of reaction in the soil. This method allows a determination of the hydrogen-ion concentration and affords an accurate measurement of the intensity of the acidity or alkalinity rather than the total acidity or alkalinity. A more rapid and a sufficiently accurate method for determining the hydrogen-ion concentration of soil extracts is the colorimetric method described by Clark and Lubs (2). Data obtained by this method agree very closely with those obtained by the electrometric method as determined by Gillespie (6) and Gillespie and Hurst (8).

In the following experiments the determinations of the hydrogen-ion concentration of the soil extracts were made by the colorimetric method and expressed in the usual terms. This method has been fully described by Clark and Lubs (2) and need not be described again. It may be added, however, that the series of standard buffer solutions employed in this investigation gave perfect agreement when compared with a corresponding series of standard buffer solutions (belonging to the Department of Bacteriology, University of Wisconsin), the hydrogen-ion concentration of which had been checked by the electrometric method. The method of obtaining the soil extracts was essentially that described by Gillespie (6).

The air-dried soil was put through a fine sieve and fifteen grams were placed in a hard glass tube of 100 cc. capacity. The soil was treated with 10 cc. of conductivity water and allowed to stand until thoroughly moist, which required about five minutes. Twenty cubic centimeters more of water was added, the tube was closed by the hand, shaken 100 times, and then allowed to stand for about five minutes while the filter was being prepared. Schleicher and Schüll ashless filter paper was used. After having been shaken 100 times as before, the contents of the tube were poured upon the filter, and filtration was accomplished with moderate suction. With care in conducting the filtration process, extracts were obtained which in

most cases were quite clear. Some, however, were more or less turbid; hence, to avoid probable errors in making color comparisons, the turbidity was reduced by dilution. Five-cc. portions were placed in test tubes of the same diameter as those containing the buffer solutions, and each portion diluted with 5 cc. of water. The indicator solution was then added, admixed, and the color resulting was compared with the colors obtained on adding the same quantity of the same indicator to 10 cc. of the various buffer solutions of known hydrogen-ion concentration. A comparator was used in making the color comparisons. Whenever possible, checks were made by using different indicators. Checks were also made by using the soil extract without dilution, but in no case were the results found to vary appreciably. Clark and Lubs (1) found that a much greater degree of dilution than was used in these tests has such a small effect on the hydrogen-ion concentration that it can seldom be detected by the colorimetric method.

Experiments with Tomato Seedlings in Infected Soil

Experiment 1

In his preliminary work Clayton (*loc. cit.*) arranged a series in which equal quantities of the two types of soil were treated with successively increasing quantities of calcium carbonate as shown by the following record:

Number of Flat	Treatment	
	Sandy Loam Soil	Silt Loam Soil
1	No treatment	No treatment
2	11.1 grams $CaCO_3$	22.5 grams $CaCO_3$
3	22.5 " "	45.0 " "
4	45.0 " "	90.0 " "
5	90.0 " "	135.0 " "
6	135.0 " "	

The treatment above described had been given the soils several months prior to the time when the writer assumed charge of the experiments. The flats used were about four inches deep, and large enough to allow fifty plants to grow for a period of from four to six weeks without too much crowding.

In addition there was added to each series a flat of soil in which an attempt was made to secure a more acid condition than that which existed in the naturally acid soils of flats no. 1. Twenty cubic centimeters of concentrated hydrochloric acid was considerably diluted, poured over the surface of the soil, and thoroughly admixed. These flats were designated 1*A*. Sterilization was accomplished by autoclaving the soils at fifteen pounds' pressure for two hours. The flats were then allowed to stand for three days, when the soils were reworked and inoculated, and Chalk's Early Jewel tomato seed was planted in rows. The planting was thick enough to insure a good stand so that the plants could be thinned out to the desired number in each flat.

In all the experiments the soil was inoculated in the following manner.

The organism was increased for inoculation purposes by being grown on steamed rice in Erlenmeyer flasks, inoculations of these cultures being made with a spore suspension from the original tube cultures. It generally required a period of about three weeks for the macroconidia to be produced in abundance, hence the flask cultures were incubated for three or four weeks. Equal quantities of a very heavy water spore suspension made from the rice cultures were poured over the surface of the soil and very thoroughly admixed. The soil had been previously allowed to dry enough so that the amount of suspension added would bring the soil to a good condition for planting.

The experiment was started on January 13. On January 28, when the plants were just putting out the first true leaves, they were thinned out to 50 plants in each flat. The experiment was continued until February 16. During this period the temperature in the greenhouse was maintained quite constantly at 26 to 28° C. Much of the time, however, cloudy weather prevailed; consequently the light relations were poor and the plants grew slowly.

Toxic effects on the growth of the plants in flats no. 1*A* were very marked, as was expected. Germination was delayed three days, growth was very slow, and none of the plants attained the size of those in the other flats. Many of the plants died from undetermined causes, though a considerable number developed typical symptoms of the wilt. On account of the difficulty in most cases of determining whether the death of the plants resulted exclusively from wilt infection, an accurate count was not attempted.

The first symptoms of wilt appeared on February 7, when several of the plants were found to have wilted during the day. When the plants were examined, the dark discoloration of the vascular system was very plainly evident. It was noted throughout the course of the experiment that preliminary symptoms of leaf yellowing were seldom manifested. Plants apparently healthy when examined in the evening would be found completely wilted by the next morning, or such wilting was sometimes found to have taken place during the day. In all cases it seemed evident from examination of wilted plants that infection occurred quite early in the life of the seedling. The primary xylem was always found to be discolored, though in many cases discoloration of the vascular system did not extend very far above the surface of the ground. A prominent symptom observed, however, was a stunted condition of the plant. For several days, usually, no other symptom was noticeable, but all such plants finally wilted, and the vascular system showed the typical discoloration.

Wilted plants were removed each day for examination. In case of any doubt as to the cause of the wilting, platings were made to determine the presence of the fungus. A few of the plants were destroyed by "damping-off" fungi or by other causes. Such plants were discarded and were not included in the total number of plants in the flats from which percentages

were figured. At the end of the experiment, by which time the plants were getting too large for good growth in the flats, all plants still standing were carefully removed from the soil and examined for wilt infection. Great care was used in this examination, and all plants showing the slightest signs of vascular discoloration were classed as infected. The results of the experiment are shown in table 1.

TABLE 1. *Influence of Soil Reaction on the Development of Fusarium Wilt of Tomato Seedlings*

Type of Soil	Number of Flat	Reaction of Soil, pH	No. Plants Died in Six Weeks	No Plants not Wilted but Found Infected	No. Plants in Flat	Percentage of Plants Diseased	Percentage of Plants Dead
Sandy loam.....	1	6.2	17	9	53		
" "	2	6.2	23	8	50	55.3	38.8
" "	3	6.6	8	10	50	47.5	28.2
" "	4	6.6	20	9	49		
" "	5	6.8	10	9	50		
" "	6	6.8	12	10	50	41.0	22.0
Silt loam.....	1	5.8	24	12	47	76.6	51.0
" "	2	6.0	7	8	43	34.9	16.3
" "	3	6.2	6	12	50		
" "	4	6.2	7	5	50	30.0	13.0
" "	5	6.4	5	7	50	24.0	10.0

At the close of the experiment soil samples were taken in order to determine the reaction, but the determinations were not made until two weeks later. It should be noted that a drop of 1.0 pH means that the acidity of the medium is increased ten times. A drop of 0.3 pH approximately doubles the acidity. The differences in reaction are thus greater than the figures in table 1 might seem to indicate. Among several of these soils no difference could be detected; consequently the flats containing soil of the same reaction are grouped together in the table. As might be expected, some variations from uniformly consistent results occurred even in soils having the same reaction. There is shown, however, a distinct gradation in the amount of wilt developing from the more acid down to the less acid soils.

Experiment 2

In preparation for this experiment the soils in all the flats, except numbers 1*A* and 1, were treated with increasing quantities of pure calcium carbonate. Beginning with flats no. 2 the quantities added were 7.5 grams, 15 grams, 30 grams, 60 grams, and 90 grams, respectively. Figured on the basis of 2,000,000 pounds as the weight of one acre of soil six and two thirds inches deep, these amounts represent the application of approximately one, two, four, eight, and twelve tons per acre. After thorough admixing was accomplished, the flats of soil were steamed for six hours at a pressure of about four pounds. After they had been allowed to stand for three days, the soils were reworked and samples taken for a determination

of the reaction. The soil was then inoculated and planted as before with Chalk's Early Jewel tomato seed.

Germination of the seed in flats no. 1*A* was apparently normal, occurring at the same time as that in the other flats. The plants grew well and appeared thrifty, though they never attained quite the size of the plants in the other flats. The reaction of the soils at the time of planting the seed, and the results of the experiment are given in table 2.

TABLE 2. *Influence of Soil Reaction on the Development of Fusarium Wilt of Tomato Seedlings*

Type of Soil	Number of Flat	Reaction of Soil, pH	No. Plants Died in Four Weeks	No. Plants not Wilted but Found Infected	No. Plants in Flat	Percentage of Plants Diseased	Percentage of Plants Dead
Sandy loam.....	1*A*	5.0	44	0	44	100.0	100.0
" "	1	5.8	22	14	49	73.4	45.0
" "	2	6.4	21	10	50	62.0	42.0
" "	3	6.8	21	9	50	60.0	42.0
" "	4	7.0	16	7	50		
" "	5	7.0	16	8	50	47.0	32.0
" "	6	7.2	9	6	50	30.0	18.0
Silt loam.....	1*A*	5.0	33	1	35	97.1	94.3
" "	1	5.6	30	18	50	96.0	60.0
" "	2	5.8	22	14	48	75.0	46.0
" "	3	6.0	13	11	47	51.0	27.7
" "	4	6.4	14	10	50	48.0	28.0
" "	5	6.8	12	20	50	64.0	24.0

It will be noted that the soil in flats nos. 4 and 5 of the sandy-loam series gave the same reaction; consequently the results were combined. The soil in all the flats tended to be somewhat more acid than in the previous tests, though, with the exception of the two flats noted, a good gradation was secured. From these tests it would appear that calcium carbonate alone is not effective in producing a marked alkaline reaction in the soil, at least unless excessive amounts are added, or unless it is allowed to act for a considerable period of time.

It is interesting to note in this connection that Plummer (18), in reporting the hydrogen-ion concentration of soils as determined by means of the hydrogen electrode, gives an example of a soil having as alkaline a reaction as pH 9.7. This soil, however, is stated to be a Cumberland loam soil which had been treated two years previously with 16,000 pounds of calcium carbonate per acre in excess of the Vietch lime-requirement indication.

The results of this experiment show again the gradual reduction in the amount of wilt developing from the acid end to the more alkaline end of the series, with one slight exception. In flat no. 5 of the silt series, the total percentage of plants found infected is 64. This is greater than that found in flats nos. 3 and 4. The percentage of plants which died, however, is somewhat less than in the others.

The experiment was carried out during the month of March. In conse-

quence of the fact that more clear weather prevailed than was the case in the preceding experiment, there was considerable fluctuation in the temperature of the greenhouse during the daytime. Occasionally the temperature ran as high as 35° C. for a time.

Experiment 3

This experiment was conducted with a series of five flats of silt-loam soil of the same kind as that used in the preceding experiments. These flats were about six inches deep and held about ten kilograms of dry soil. The series was arranged as follows:

Flat no. 1 received no treatment of calcium carbonate; flat no. 2 received 60 grams, or approximately five tons per acre; flat no. 3 received 120 grams; flat no. 4 received 60 grams, and an additional application of 90 grams of freshly slaked pure calcium oxide; flat no. 5 received 60 grams of the carbonate and 180 grams of the slaked oxide.

The flats were placed in the autoclave and subjected to fifteen pounds' pressure for two hours. Two days later the soil was inoculated and planted as usual. About the beginning of the first true leaf stage the plants were thinned out to 30 in each flat. The reaction of the soil and the results of the experiment are shown in table 3.

TABLE 3. *Influence of Reaction in Silt-Loam Soil on the Fusarium Wilt of Tomato Seedlings.*

Number of Flat	Reaction of Soil, pH	No. Plants Died in Four Weeks	No. Plants not Wilted but Found Infected	No. Plants in Flat	Percentage of Plants Diseased	Percentage of Plants Dead
1	6.0	15	9	30	80.0	50.0
2	6.4	8	10	30	60.0	26.6
3	7.0	4	4	30	26.6	13.3
4	7.4	0	3	30	10.0	0
5	8.2	0	0	30	0	0

In this series an excellent gradation of the soil reaction was secured, that in flat no. 5 being markedly alkaline. It must be stated, however, that the plants in this flat made a very poor growth. Germination occurred promptly, but the plants lagged in growth, were very dark green in color, and presented a generally unthrifty appearance. Every plant lived, however, and no indication of wilt infection could be detected when they were pulled and examined. The time of this experiment was coincident with that of experiment 2, hence the same fluctuations of temperature occurred as were mentioned in connection with that experiment.

Experiment 4

In preparation for this experiment the flats of soil that were used in experiment 2 were rearranged as follows:

Sandy-loam series. Flats no. 1*A*, no. 1, no. 3, no. 5, and no. 6 were re-

numbered 1, 2, 3, 4, and 5 respectively. The old flat no. 2 was discarded. The soil in the old flat no. 4 was treated with 60 grams of calcium carbonate, 50 grams of slaked calcium oxide, and 10 grams of magnesium sulphate. This flat was added to the series and numbered 6.

Silt-loam series. Flats no. 1*A*, no. 1, no. 3, and no. 5 were renumbered 1, 2, 3, and 4 respectively. The soil in the old flat no. 2 was treated with 70 grams of calcium carbonate, 50 grams of slaked calcium oxide, and 10 grams of magnesium sulphate. The soil in the old flat no. 4 was treated with 60 grams of calcium carbonate, and 10 grams of magnesium sulphate. These flats were added to the series and numbered 5 and 6 respectively.

The soil in all the flats was given an application of a 6–16–8 fertilizer (composed of calcium nitrate, potassium chloride, and monocalcium phosphate) at the rate of about 600 pounds per acre. The soils were not sterilized, but were reinoculated and planted as before. The results obtained are given in table 4.

TABLE 4. *Influence of Soil Reaction on the Development of Fusarium Wilt of Tomato Seedlings*

Type of Soil	Number of Flat	Reaction of Soil, pH	No. Plants Died in Five Weeks	No. Plants not Wilted but Found Infected	No. Plants in Flat	Percentage of Plants Diseased	Percentage of Plants Dead
Sandy loam	1	5.4	28	20	50	96	56
" "	2	5.8	15	15	50	60	30
" "	3	6.4	11	8	50	38	22
" "	4	6.8	12	9	50	42	24
" "	5	7.2	1	5	50	12	2
" "	6	8.2	3	8	50	22	6
Silt loam	1	5.6	39	5	50	88	78
" "	2	5.8	28	13	50	82	56
" "	3	6.0	4	12	50	32	8
" "	4	6.4	13	20	50	66	26
" "	5	6.8	9	13	50	44	18
" "	6	7.2	5	16	50	42	10

The development of the disease was much slower in this experiment than in those preceding, in which the soil was sterilized before inoculation. The first symptoms did not appear until three weeks after planting. In many cases wilting of only a portion of the plant was observed for two or three days before general wilting occurred. Preliminary leaf yellowing was also noticed in a number of cases. The plants grew more rapidly than in the other experiments, though considerable cloudy weather prevailed. On clear days the temperature fluctuated in a manner similar to that previously described.

As in the preceding experiments there was a considerable reduction in the number of diseased plants occurring at the alkaline end of each series. The plants produced in the sandy soil at pH 8.2 were hardly as large as those produced at 7.2, but they presented a very thrifty appearance. In general

appearance they were very similar to those in flat no. 1 which contained the most acid soil in the series.

In flat no. 3 of the silt-loam series a very low percentage of infection occurred. This may be due in part, at least, to the fact that the soil in this flat was somewhat drier than in the others at the time of planting the seed. The conspicuous contrast in the condition of the plants in flats nos. 1 and 5 of the sandy-loam series is shown in Plate XXXVIII. The photographs were taken on May 7 just before harvesting.

Experiments with Fusarium on Culture Media

Experiment 1

These experiments were designed with the view of studying spore germination and mycelial growth of *Fusarium lycopersici* in culture media adjusted to various hydrogen-ion concentrations. The purpose of the experiments was to determine whether under different reactions any correlation exists between the growth of the fungus in culture and its behavior in soils as expressed by the development of the disease in the plants.

After some preliminary experiments in attempting adjustments of various liquid media, it was decided to use a medium made from "Difco" peptone, 1.0 percent; dibasic potassium phosphate, 0.5 percent; dextrose, 3.0 percent; and distilled water, 1000 cc. Tests showed that the fungus would grow well when this medium was adjusted to plus 1, Fuller's scale. Moreover, it was found that adjustment could be made from pH 1.2 to 10 without the presence of any precipitate. Also, the natural color could be practically destroyed by one-half dilution with water, making the colorimetric determinations an easy matter with the use of the comparator.

Culture flasks were prepared in duplicate. Two hundred fifty cubic centimeters of the medium was placed in Erlenmeyer flasks of about 300 cc. capacity and adjusted with additions of sodium hydroxide or hydrochloric acid to the desired hydrogen-ion concentration. After the adjustment was made, 25 cc. was removed and placed in tubes to be tested after sterilization in order to determine the final reaction. The medium remaining in the flasks was then divided into two equal portions in flasks for inoculation. Culture flasks and tubes were sterilized together at seven pounds' pressure for thirty minutes. For inoculation purposes a water spore suspension was prepared from a pure culture of the organism on oat agar. One cubic centimeter of this suspension was placed in each flask with a sterilized pipette.

The cultures were incubated in the greenhouse where the tomatoes were growing, and thus exposed to the same temperature. The flasks were kept covered so they would not be exposed to strong light. After a period of fifteen days the relative amount of growth was noted. The contents of the flasks were then filtered, with moderate suction, through the Schleicher and Schüll ashless filter paper, and the hydrogen-ion concentration of the fil-

trate was determined in order to compare the changes in the reaction of the media due to the growth of the fungus. The results are given in table 5.

TABLE 5. *Influence of the Reaction of Culture Medium on the Growth of Fusarium lycopersici*

No. of Flask	Initial Approximate Adjustment of Medium	H-ion Concentration after Sterilization	Relative Growth in 15 Days	H-ion Concentration of Medium after 15 Days
	pH	pH		pH
1	2.0	1.8	No growth	1.8
2	3.0	2.8	Heavy	3.0
3	4.0	4.4	Very heavy	4.4
4	5.0	5.0	Heavy	4.6
5	5.6	6.0	Moderate	4.8
6	6.2	6.4	Moderate	5.4
7	7.0	7.0	Moderate	5.6
8	7.6	7.6	Light	5.6
9	8.2	8.2	Light	5.0
10	9.0	8.4	Moderate	5.8

It will be noted from the table that relatively small changes occurred in the pH value as a result of sterilization. No germination occurred at pH 1.8. A moderate growth was produced from pH 6.0 to 8.4, accompanied by considerable changes toward greater acidity. The heaviest growth of mycelium took place at pH 2.8 to 5.0, without much change in the reaction of the medium.

Experiment 2

The culture medium was made and adjusted as in experiment 1. In this case, however, the culture flasks were sterilized at five pounds' pressure for twenty minutes on two successive days. They were then inoculated and incubated in the greenhouse as before. The period of incubation was coincident with the time of the growth of the plants in experiment 3, hence the cultures were subjected to the same temperature changes as were the plants. The results of the experiment are shown in table 6.

TABLE 6. *Influence of the Reaction of Culture Medium on the Growth of Fusarium lycopersici*

No. of Flask	Initial Approximate Adjustment of Medium	H-ion Concentration after Sterilization	Relative Growth in 15 Days	H-ion Concentration of Medium after 15 Days
	pH	pH		pH
1	2.0	2.2	Very light	2.2
2	3.0	3.6	Light	3.0
3	4.0	4.4	Light	3.4
4	5.0	5.0	Moderate	4.2
5	6.0	6.2	Moderate	5.2
6	6.8	6.6	Light	5.0
7	7.4	7.0	Light	5.0
8	8.2	7.2	Heavy	6.4
9	8.8	7.6	Heavy	6.6
10	9.6	8.0	Heavy	6.6

The sterilization process caused greater changes in the reaction of the medium than in experiment 1. This was especially marked in flasks 8, 9, and 10, which contained the most alkaline medium. It was also observed that the medium in these flasks had considerably more color than the others. While the heaviest production of mycelium occurred in the media testing pH 7.2, 7.6, and 8.0, growth was accompanied as before with marked changes toward greater acidity. Growth at pH 2.2 was very light in this case, and, as no germination occurred at pH 1.8 in experiment 1, it appears that under the conditions of the experiments the limiting hydrogen-ion concentration for spore germination is about pH 2.0. As far as mycelial growth is concerned, the results of the two experiments are not very consistent; but, as growth in the duplicate flasks was quite uniform in all cases, the results may be partially explained as being due to different nutritive relations caused by the sterilization process.

Discussion and Conclusions

That soil reaction has a marked influence on the development of the Fusarium wilt of tomato seedlings is clearly shown by the experimental data. The percentage of diseased plants was in all cases greatest in the most acid soils, and always decreased quite uniformly as neutrality was approached. These results have been consistent with both the sandy-loam and the silt-loam soils. The amount of infection has, however, shown considerable variation under the conditions of the several experiments. It is apparent, therefore, that the total amount of disease which developed in each experiment depended upon conditions more influential than the reaction of the soil. Thus, in the sandy-loam soil having a reaction of pH 6.8 in each experiment, the total percentage of infected plants in experiment 1 was 41; in experiment 2, 60; and experiment 4, 42. Likewise, in the silt-loam soil having a reaction of pH 6.4 in each experiment, the total percentage of infected plants in experiment 1 was 24; in experiment 2, 48; in experiment 3, 60; and experiment 4, 66.

The amount of disease developing in the two types of soils of the same hydrogen-ion concentration under the conditions of the same experiment also varies to a considerable extent. In experiment 1, for example, in soils having a reaction of pH 6.2, 55.3 percent of the plants in the sandy-loam soil became infected, of which 38.8 percent died, while in the silt soil 30 percent became infected and 13 percent died. On the other hand, in experiment 2 in soils testing pH 5.8, the corresponding figures are 73.4 percent and 45.0 percent in the sandy soil, and 75 percent and 46 percent in the silt soil. A difference may be expected in the disease development in two soils as widely different as a sandy loam and a heavy silt loam. Such factors as supply of plant food, aëration, and organic-matter content, no doubt, either directly or indirectly may play some part in influencing results.

It may be observed that it was necessary to add more calcium carbonate

or oxide to the silt soil than to the sandy soil in order to produce the same changes in reaction. The difficulty of obtaining any marked alkaline reaction with the carbonate has already been mentioned. It was even necessary to add what would ordinarily be considered excessive amounts before neutrality could be produced. Sharp and Hoagland (19) have observed that

> Some soils whose solutions are neutral or alkaline remove considerable quantities of calcium hydroxide from the solution without materially increasing the OH-ion concentration of the soil suspension. In certain cases this reaction might be erroneously attributed to soil acids.

It seems evident, therefore, that the actual reaction produced in the soil should be the basis for forming conclusions concerning the effects of liming soils, rather than the uncertain reaction produced by some amount of lime added as calculated by means of the various lime-requirement methods.

Judging from examinations of infected plants and the rapidity with which the disease develops, the critical period of infection in the seedlings appears to occur within the first few days following germination. The primary xylem was always found to be affected, and, as the result of this observation in experiment 1, it was decided to determine the reaction of the soil at planting time in the later experiments.

A difference in size was the most conspicuous variation in plants grown at pH 5.6–5.8 to 7.0–7.4; the smallest plants were produced in the acid soils. A very uniform gradation occurred until the neutral point was approached. At this point the largest plants were produced. At hydrogen-ion concentrations greater than about pH 5.6, germination was slightly retarded and somewhat less uniform. Usually the plants produced in the more acid soils were slightly lighter green in color, but this condition was not consistent. These variations in growth appeared to be due more to differences in nutrition than to the direct effect of acidity. It is well known that the general fertility of acid soils is affected favorably by the use of calcium carbonate. In this connection also, Truog (21) believes that "it is usually because the actual lime needs of the plant are not fulfilled that soil acidity exerts a specific injurious influence on certain plants, and not because the acidity is directly toxic or destructive."

Observations on the growth of plants in alkaline soils were very limited, since in only two cases was any degree of alkalinity secured. It will be recalled that the plants in flat no. 5, experiment 3, made a very poor growth in soil testing pH 8.2. The leaves were very dark green and the stems were very small and woody. In this case the detrimental effects may have been due in part to an excess of lime without sufficient magnesium. It is improbable that the effects were caused by soil sterilization, since plants were normal in the other flats of the series. In experiment 4 the growth of the plants was considerably slower in the sandy soil testing pH 8.2 than at pH 7.2. It is interesting to note in this general connection that Hoagland's

(11) experiments with barley seedlings grown in nutrient solutions showed a reaction of pH 8.3 to be distinctly injurious to the growth of the plants.

The experiments with the growth of the organism in culture should be considered merely preliminary to a complete study of the effect of the hydrogen-ion concentration of culture media on the growth of the fungus. It is clearly understood that a complete study would require frequent testing during the arbitrarily selected fifteen-day period to determine the immediate changes in the reaction due to the growth of the fungus. Moreover, it is not known what further changes might have taken place after the fifteen-day period. The results of the two experiments as conducted, however, seem to justify the conclusion that the fungus is able to grow within the range of hydrogen-ion concentrations which most likely occur in ordinary soils. Furthermore, the limiting acid reaction for spore germination under the conditions of the experiments seems to be very well established at approximately pH 2.0.

Summary

(1) In conducting experiments to determine the effect of soil reaction on the development of plant diseases caused by soil-infesting organisms, it is necessary to know the result actually produced by materials used to bring about changes in the reaction.

(2) The colorimetric measurement of the hydrogen-ion concentration of soil extracts affords a means of accurately determining the reaction of the soil before and after such materials are added. Thus, there can be arranged a series of soils from strongly acid to alkaline, the exact reaction of each member of which is known.

(3) Experiments were conducted with naturally acid silt-loam and sandy-loam soils, adjusted to various degrees of acidity and alkalinity by the use of calcium carbonate and calcium oxide, in order to determine the effect of soil reaction on the development of Fusarium wilt of tomato seedlings.

(4) The highest percentage of wilt always occurred in the most acid soils of the series. With very few exceptions, the percentage of wilt decreased quite uniformly as the hydrogen-ion concentration of the soils decreased, until approximately pH 7.4 was reached. The percentage of wilt which developed in soils having a more alkaline reaction than pH 7.4 was lower in one case and higher in the other, but the plants made a very poor growth in soils having a reaction more alkaline than pH 7.4. No sharp delimitation was apparent, neither could any limiting degree of acidity or alkalinity be found at which the disease would not develop.

(5) Culture experiments were carried out with *Fusarium lycopersici* in nutrient solutions adjusted to hydrogen-ion concentrations ranging from pH 1.8 to 8.4. Spores of the organism exposed to the same temperatures as the growing tomato seedlings germinated in the solutions varying in hydrogen-ion concentrations from pH 2.2 to 8.4. No germination occurred in the solutions adjusted to pH 1.8. Growth of the fungus in the solutions ad-

justed to pH 2.2 was very light and caused no change in the reaction of the medium. The fungus grew well at all hydrogen-ion concentrations from pH 2.8 to 8.4. Growth at pH 2.8 in one case caused a slight change in the reaction of the medium toward greater alkalinity. At all hydrogen-ion concentrations from pH 3.6 to 8.4, the growth of the organism was accompanied by changes toward greater acidity.

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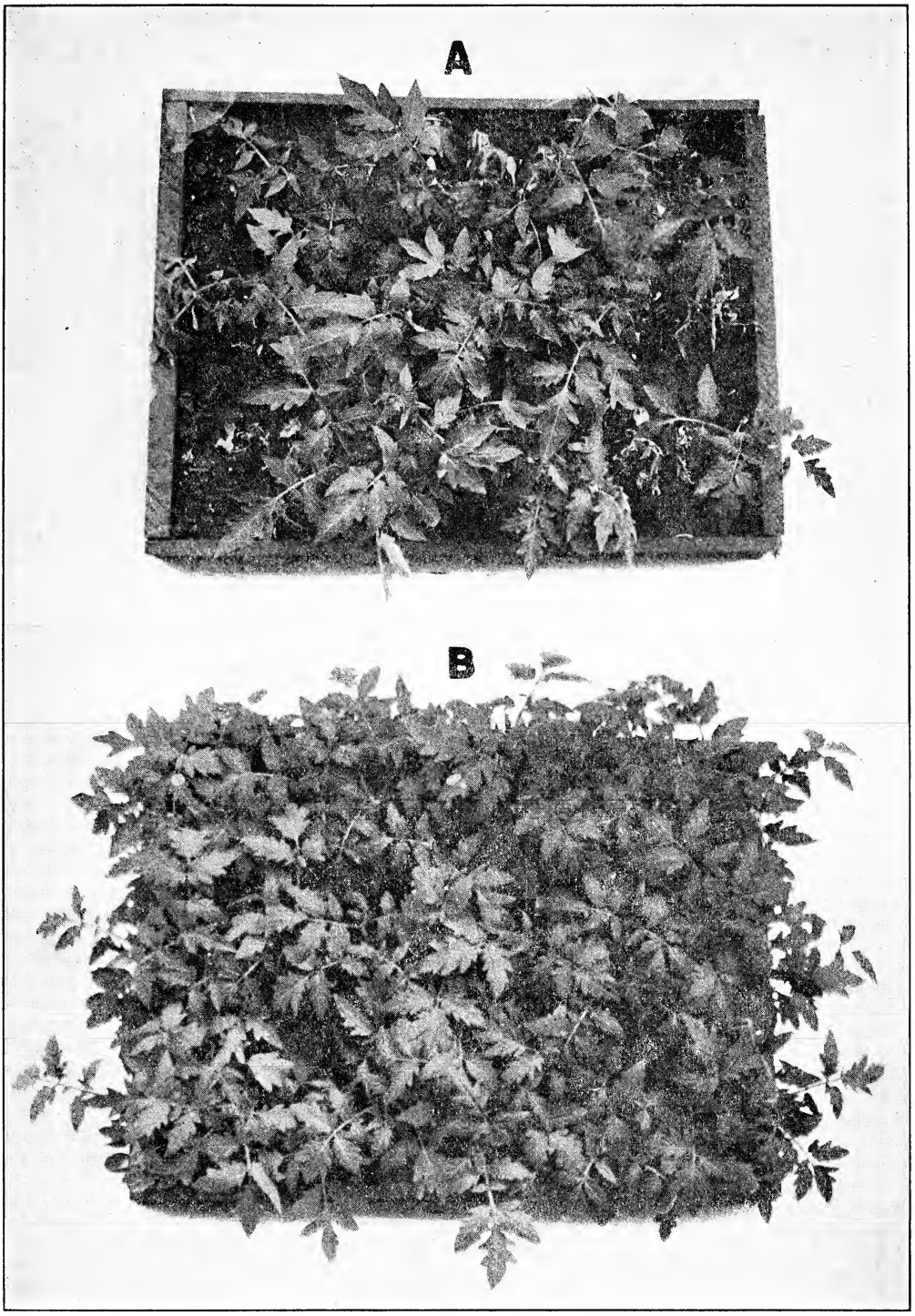

SHERWOOD: FUSARIUM WILT OF TOMATO

EXPLANATION OF PLATE XXXVIII

In the upper flat, *A*, in the acid soil, nearly every plant (96 percent) showed infection, and over one half (56 percent) died.

In the lower flat, *B*, in the alkaline soil, only 6 seedlings out of 50 showed infection, only two plants died.

For further details consult table 4 and accompanying text.

A. Sandy-loam soil, experiment 4, flat no. 1. This shows the appearance of the seedlings at the end of five weeks' growth in Fusarium-infected soil testing pH 5.4. Total percentage of infection, 96; percentage of plants that died, 56.

B. Same sandy-loam soil, experiment 4, flat no. 5. This shows the appearance of the seedlings at the end of five weeks' growth in Fusarium-infected soil testing pH 7.2. Total percentage of infection, 12; percentage of plants that died, 2.

STUDIES ON THE RELATION OF AERATION AND CONTINUOUS RENEWAL OF NUTRIENT SOLUTION TO THE GROWTH OF SOYBEANS IN ARTIFICIAL CULTURE[1]

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Introduction

A study of the literature upon the relation between plant growth and oxygen pressure of the root environment shows that this relation is not a simple one owing to the complexity of the many variable factors involved. On the basis of our present knowledge it would be difficult to specify what should be the conditions, with respect of oxygen pressure, for optimum growth of a particular species in a given medium. It would be impossible, on this basis, to specify optimum conditions for plants in general, since the requirement of plants for oxygen in the sub-aërial environment has been found so distinctly variable among different species.

The study here considered has been carried out for the purpose of further investigating the need of oxygen through aëration in the root environment with special reference to plants grown in sand and solution cultures, and to study in particular the effectiveness of a continuous flow of nutrient solution and constant aëration of the substratum in meeting this requirement. During the progress of the work the importance of considering the oxygen supply of the root environment in plant studies became apparent when it was observed how rapidly plants under favorable growth conditions are capable of exhausting the substratum of its store of dissolved oxygen, this being a matter of hours rather than of days when the roots of plants in a culture are surrounded by only a liter of nutrient solution which is not constantly aërated.

Procedure

The plan of the present experiment, briefly stated, involved a study of the growth of soybean plants (variety Manchu) in both solution and sand cultures under four distinctly different types of treatment as outlined below. Seed of high purity was obtained for this work through the courtesy of the Johnson Seed Farms, Stryker, Ohio. In the arrangement of the series, duplicate cultures of each of the four treatments, carrying three

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plants each, were placed both in solution and in sand. The nutrient solution used for all cultures was that found by Shive (7) to be well adapted for the growth of soybeans. This solution was used at a total concentration of 1.75 atmospheres and contained the three salts KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ in the proportions of .00406 M, .0034 M, and .0225 M, respectively. As a source of iron, one milligram of the element in the form of ferrous sulphate was used per liter of solution for all cultures.

The four treatments accorded the plants in both sand and solution cultures may be outlined as follows:

Series I. Simple non-aërated cultures. Solutions renewed every three and one half days.

Series II. Simple aërated cultures. Like I, but aërated by a continuous bubbling of air through the medium.

Series III. Drip cultures. Solutions continuously renewed, but not aërated.

Series IV. Aërated-drip cultures. Like III, but aërated by a continuous bubbling of air through the medium.

For all solution cultures quart jars were used in the manner described by Shive (6) and adapted to the various treatments. For those cultures not receiving a constant drip the period between solution renewals adopted for both solution and sand cultures was three and one half days.

For the sand cultures percolators were prepared by cementing together, after removing the bottoms from all except the smallest, a series of four ordinary cone-shaped clay pots, the largest of which was 23 cm. in the upper diameter and the smallest 7.5 cm. In cementing the pots together, a fairly thin mixture of Portland cement was used, the bottom of the larger being fitted into the top of the smaller consecutively, the bottom of the smallest pot forming the bottom of the cone-shaped vessel thus constructed. These percolators were 50 cm. in height and were capable of holding nine kilograms of dry sand. After air-drying they were heated in a hot-air sterilizer for the purpose of removing hygroscopic moisture preparatory to treating them both inside and outside, while still warm, with hot paraffin. This treatment was found to prevent the penetration of the walls by the solution. The opening through the bottom of each percolator was closed by a rubber stopper carrying a short glass tube through which the percolating solution could escape. The upper end of this tube was loosely closed with a small plug of glass wool.

Each vessel when completed was filled with nine kilograms of clean white sand which had been washed with tap water followed by distilled water. All the sand cultures employed in the experiments, whether solutions were renewed continuously or only intermittently, were prepared in these culture vessels.

For sand-culture work in which the nutrient solutions are continuously renewed by percolation through the sand, a deep vessel having a form somewhat like those here described is desirable if not an actual necessity in order to prevent excessive moisture in the median and upper portions of the sand

culture where the roots of the plants are mainly developed. Tests of the moisture content of the sand in the culture vessels here used, when one liter of solution percolated through this substratum in 24 hours, showed that in the bottom of the vessel the moisture content of the sand approached saturation, while in the median regions it approximated 60 percent of the maximum water-retaining capacity and decreased progressively toward the surface.

Aëration in both solution and sand cultures was accomplished by means of the improvised air pump previously described and illustrated (1). By this means a continuous stream of air bubbles was passed through both the solution and the sand cultures of series II and IV, the point of entrance of the air in both cases being near the bottom of the culture vessel.

A brief discussion of the question of maintaining a continuous flow of the nutrient solution through the culture vessel has been presented recently by Trelease and Livingston (8), who have described a satisfactory apparatus for obtaining such a flow. In their argument for the desirability of such a modification in the customary methods of studying the salt requirement and concentration relations in culture solutions, they have considered the comparatively prompt change in the concentration and chemical make-up of such solutions when brought into contact with the roots of rapidly growing plants. Further argument in favor of the continuous renewal of the mineral nutrients in solution cultures might well be based upon a consideration of reaction change brought about in the solutions by contact with the plant roots, and of the aëration which such renewal affords. Both of these factors will be emphasized.

The apparatus here used, by means of which a continuous renewal of the nutrient solutions was accomplished, is diagrammatically represented in figure 1. This apparatus consists of a solution reservoir, a constant-level reservoir, and a main conducting tube *C* with its branches leading to the cultures. The constant level as indicated in figure 1 is found to fluctuate within a range of ½ to 1 cm., depending upon the quantity of solution in the upper reservoir at any given time. It is readily seen that, as the level drops away from the opening of the air inlet *A*, the equilibrium through the tube is broken and air enters the solution reservoir and the solution drops through *B* until the level in the lower vessel rises over the opening of the air inlet *A*, and equilibrium is again restored. In siphoning from the constant-level reservoir through the main tube *C* to the cultures, the rate of flow must necessarily be controlled, since, as above stated, it was desired to deliver approximately one liter of solution per day per culture. It has been found repeatedly that the rate of solution flow can not be controlled satisfactorily by the use of metal clamps on rubber connections. In order to obtain the required rate of solution flow through the culture, a capillary tube of one-half-millimeter bore was used to carry the solution from the conducting tube *C* to the culture, the rate of flow being regulated by the length of this capil-

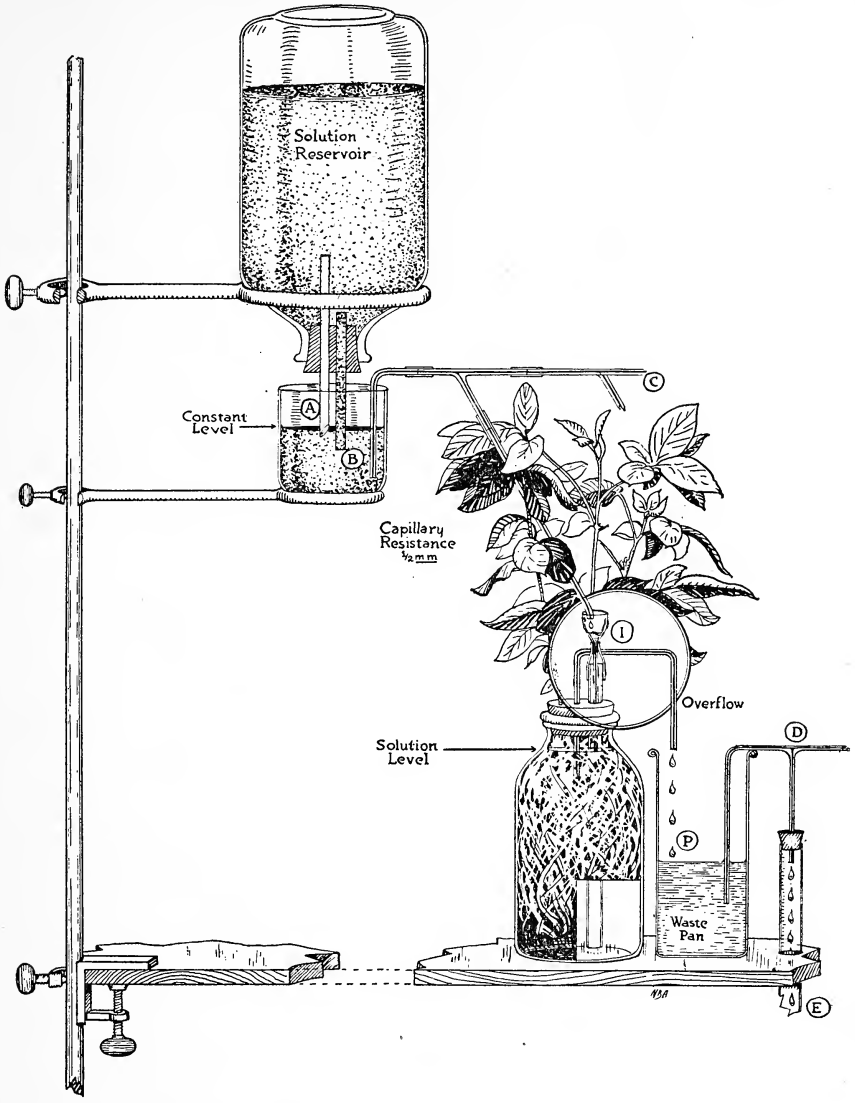

FIG. 1. Diagram of apparatus used for continuous renewal of nutrient solution.

lary resistance and the elevation of the constant level above the outlet of the siphon thus formed. Any desired rate of flow can readily be obtained in this manner by a few trials. Experience has shown that these capillary tubes do not readily clog as do rubber tubes upon which metal clamps are set, and by their use a fairly uniform flow can be maintained with only an occasional cleaning. It should be noted that the main line *C* is composed of glass tubing having a bore of 4–6 mm. or larger, so as to avoid appreciable resistance to solution flow in this part. The more permanent parts of the exposed tubes were painted black to prevent the growth of algae.

At the point of outlet from the capillary tube the solution drips directly into the small funnel *I* upon which the tube rests, and passes to the bottom of the culture vessel through the glass tube provided for that purpose. It is possible with a close-fitting rubber connection to raise or lower this funnel at will, thus regulating the length of the siphon and consequently the rate of flow. In the non-aërated cultures of series III it was found necessary to use special care in preventing the inflowing solution from entraining air bubbles and carrying them to the bottom of the culture vessel, thus setting up in a small way an aërating system. This was prevented readily by the use of an inlet tube of larger diameter and by receiving the drip on the side of the funnel rather than permitting it to fall directly into the opening.

The overflow from the culture jar was provided for by means of an automatic siphon suggested and used by Dr. Shive. This siphon, shown in the diagram of figure 1, consists of a bent glass tube having a bore of about 2.0 mm., with one arm of the tube about 1.5 cm. longer than the other. The siphon is held in position by the paraffined cork stopper which supports the seedlings. The long arm of the siphon extends just below the surface of the solution, which lies in a horizontal plane passing through the short arm at a point just above the outlet. When the system is in operation, the siphon having been started, the solution surface in the jar may be maintained automatically at any level desired by raising or lowering the siphon. The overflowing solution may be caught in a waste jar or may be disposed of in any convenient way.

Continuous renewal of solution in the sand cultures was accomplished by allowing the solution to drip directly upon the sand in the center of the vessel. A watch glass was inverted just beneath the surface of the sand at this point for the purpose of spreading the solution as it percolated downward to the outlet tube through the bottom of the vessel. The rate of solution flow in the sand cultures, as in the solution cultures, was maintained at approximately one liter per day per culture.

Seeds were germinated in moist sphagnum moss, and carefully selected seedlings when about 5 cm. tall were transferred to the prepared culture media (solutions and sand) on August 16, 1922. The plants were grown during a period of 62 days and were harvested on October 17.

Experimental Results

Sand Cultures

In table 1 are given the relative dry weights of the plants grown in the sand cultures. Each value represents the average of plants or plant parts of two like cultures and is based upon the corresponding dry-weight value of the simple sand cultures as 1.00. Examination of table 1 shows that aëration has no beneficial effect upon the growth of tops as compared with that of the plants in the simple sand cultures. It does, however, produce con-

siderable increase in root development. The plants in both the simple and the aërated cultures were entirely normal in so far as this could be determined by observation. It will be observed that the relative dry-weight values of the plants grown in the drip cultures are in every instance much superior to the corresponding values for the simple cultures, the total

TABLE 1. *Relative Dry Weights of Plants Grown in Sand Cultures*

Cultures	Tops				Roots	Total Dry Weights
	Stems	Leaves	Fruit	Total		
Simple solution	1.00* (5.520)	1.00 (9.257)	1.00 (21.831)	1.00 (36.607)	1.00 (2.253)	1.00 (38.861)
Simple aërated solution	1.019	.950	.953	.963	1.078	.970
Drip solution	1.218	1.154	1.260	1.254	1.331	1.233
Aërated-drip solution	1.718	1.667	1.455	1.548	1.738	1.559

* The dry-weight values of the simple solution cultures are taken as 1.00 and corresponding values of the other cultures are expressed in terms of these. The actual dry weights of the simple solution cultures are given in parenthesis, in grams.

weight of tops being 25.47 percent and that of roots 33.1 percent higher than the corresponding weights of the plants grown in the simple sand cultures. The most pronounced superiority, however, is shown for the aërated-drip cultures, the average relative yields of tops and roots from these cultures being 54.8 percent and 73.8 percent higher, respectively, than the corresponding ones of the simple cultures. The aërated-drip cultures are thus shown to be as much superior to the non-aërated drip cultures as the latter are superior to the simple cultures.

It is apparent that constant aëration in connection with continuous solution renewal exerted a pronounced accelerating influence upon the growth rates of both tops and roots, while in connection with the simple cultures (in which solutions were renewed only every three and one half days) no such influence is observed. The explanation of this discrepancy is not at all clear. It might be suggested, however, that the infrequency of solution renewal in the case of the simple cultures may introduce a growth-retarding factor which aëration alone can not correct, but which is entirely eliminated by continuous solution renewal, and in the absence of this factor aëration may exert a powerful accelerating influence upon the growth rates of the plants. That such a growth-retarding factor is actually introduced by infrequency of solution renewal is clearly brought out by a comparison of the yield values from the simple sand cultures with those from the constant drip cultures (table 1).

The general appearance of the plants of this experiment with respect to size, health, and vigor, together with the data presented in table 1, shows that constant aëration in connection with continuous solution renewal by

percolation through the sand medium provides unusually favorable conditions for the complete development of the plants.

In the constant-drip cultures and in the aërated-drip cultures it was found that the roots had penetrated to the bottoms of the culture vessels, notwithstanding the nearly saturated condition of the substratum in this region, while in the simple cultures and in the aërated cultures root development in the lower portions of the substratum was much less pronounced.

Table 2 gives the ratios between dry weights of the different plant parts and the total dry weights of the plants which are given in the last column of the table, the values, of course, representing averages in each case. As indicated in the table, the total average dry weights of the plants grown

TABLE 2. *Ratios of Average Dry Weights of Plant Parts to Average Total Dry Weights of Plants Grown in Sand Cultures*

Cultures	Tops				Roots	Total Dry Weights
	Stems	Leaves	Fruit	Total		
Simple solution	0.142	0.238	0.562	0.942	0.058	38.861
Simple aërated solution	.149	.234	.553	.936	.064	37.687
Drip solution	.140	.223	.574	.937	.063	47.913
Aërated-drip solution	.157	.254	.524	.935	.065	60.581

under the several experimental conditions show large differences. On the other hand, the development of the plant parts relative to the total yields produced under the different experimental treatments is strikingly similar, as is indicated by the pronounced uniformity of corresponding ratio values.

Solution Cultures

Table 3, which is similar to table 1, gives the numerical data of the plants grown in solution cultures corresponding to the sand cultures previously described. From a comparison of the data for simple solution cultures with those of the aërated solution cultures, it will be observed that with these, as with the corresponding sand cultures, aëration produced no

TABLE 3. *Relative Dry Weights of Plants Grown in Solution Cultures*

Cultures	Tops				Roots	Total Dry Weights
	Stems	Leaves	Fruit	Total		
Simple solution	1.00* (6.386)	1.00 (8.876)	1.00 (4.640)	1.00 (19.896)	1.00 (2.324)	1.00 (22.220)
Simple aërated solution	.983	1.064	. . .	.776	1.117	.812
Drip solution	.815	.952	3.775	1.567	.743	1.503
Aërated-drip solution	1.412	1.296	5.392	2.339	1.433	2.199

* The dry-weight values of the simple solution cultures are taken as 1.00 and corresponding values of the other cultures are expressed in terms of these. The actual dry weights of the simple solution cultures are given in parenthesis, in grams.

beneficial effects except upon the development of roots. The accelerating influence of aëration on root development, however, is quite pronounced both in sand and in solution cultures. The superiority of the roots grown in the aërated solutions over those in the simple solution cultures is manifested not so much by the higher dry weight of root substance produced as by the development of a very efficient absorbing system. The main roots in the aërated cultures were long and slender and thickly beset with well developed laterals almost to the very tips, thus giving rise to large and efficient absorbing surfaces. This response to aëration, however, was much more pronounced in the culture solutions which were continuously renewed than it was in those which were only intermittently renewed (every 3½ days). The superior root growth resulting from aëration in the drip cultures is particularly reflected in accelerated top growth during the later stages of development. This is clearly brought out in the photographs of Plate XXXIX, which show an aërated and a non-aërated drip culture when the plants were 14 days old (above), and the same cultures at the age of 37 days (below). The data of table 3 show that the dry weights of stems, leaves, and roots from the drip-solution cultures without aëration were inferior to those from the simple solution cultures, although the average total dry weights of the former were more than 50 percent higher than those of the latter, this superiority of total yield being due entirely to the remarkable fruit-production by the plants grown in the continuously renewed solutions.

In the solution cultures, as in the sand cultures, constant aëration of the medium in connection with continuous solution renewal produced by far the highest yields throughout, the average total dry weights of tops and of roots being 133.9 percent and 43.3 percent higher, respectively, than the corresponding yields from the simple solution cultures. The average dry weight of tops from these aërated-drip cultures was greatly augmented by the exceptionally high average yield of fruit, which was approximately one half the average total dry weight of the plants.

The ratios of average dry weights of plant parts to the average total dry weights are given in table 4, this table corresponding, item for item, with table 2 relating to sand cultures.

TABLE 4. *Ratios of Average Dry Weights of Plant Parts to Average Total Dry Weights of Plants Grown in Solution Cultures*

Cultures	Tops				Roots	Total Dry Weights
	Stems	Leaves	Fruit	Total		
Simple solution	0.287	0.399	0.209	0.895	0.105	22.220
Simple aërated solution	.333	.523	. . . .	.856	.144	18.047
Drip solution	.157	.257	.532	.946	.054	32.900
Aërated-drip solution	.185	.235	.512	.932	.068	48.869

As is indicated by the lack of agreement between corresponding ratio values, there is a marked difference between the plants grown in the simple solution cultures and those grown in the simple aërated solution cultures with respect to the distribution of total yield values among the various plant parts (stems, leaves, fruit, and roots). Corresponding ratio values relating to the drip cultures and the aërated-drip cultures, however, show fairly close agreement, thus indicating that the development of the plant parts relative to the total yields produced is similar under these experimental treatments but is quite different in this respect from the development of the plant parts in the simple cultures and the simple aërated cultures. A comparison of the corresponding data of tables 2 and 4 brings out the fact that this relation of average partial yields (stems, leaves, etc.) to average total yields of the plants grown in the drip-solution cultures, both aërated and non-aërated, is quite in agreement with that shown for the plants grown in the sand cultures under the various experimental treatments. It is interesting here to note also that the average total yields obtained from the sand cultures were throughout uniformly much higher than were those obtained from the solution cultures.

Oxygen Relations

Quantitative determinations of the dissolved oxygen content of the culture solutions were made at the time of solution renewal of those cultures not receiving constant drip after the solutions had been in contact with the plant roots for a period of three and one half days. No oxygen determinations were made upon the solutions in the sand cultures owing to the difficulty of collecting solution samples from these cultures without undue exposure to the atmosphere, which, of course, would have rendered tests upon such samples worthless in so far as they could have represented the true oxygen content of the solutions in contact with the plant roots at the time of sampling. The micro-Winkler method proposed by Lund (3) was used in making all the determinations.

From a large number of oxygen determinations, the results of those made during the second, fourth, sixth, and eighth weeks of the growth period are given in table 5 in terms of parts per million. These values are representative and show the general effect of the plants upon the solutions with respect to the supply of dissolved oxygen under the various experimental treatments and at different stages of the development of the plants.

From the data of table 5 it will be observed that after the second week of the growth period the oxygen content of the solutions not aërated was nearly exhausted by the plants during a three and one half day period. The oxygen content of the solutions which were continuously renewed but not aërated was also greatly reduced. This indicates that a constant drip by which one liter of fresh solution is supplied during each 24-hour period does not keep up the oxygen supply of the cultures which is necessary for maxi-

TABLE 5. *Effect of Soybean Plants upon the Oxygen Content of the Nutrient Solutions under the Various Experimental Conditions*

Cultures	Oxygen Content (p.p.m.)*			
	2nd week	4th week	6th week	8th week
Simple solution	4.75	1.25	1.00	1.75
Simple aërated solution	7.00	6.75	7.50	8.00
Drip solution	6.95	3.75	2.95	3.00
Aërated-drip solution	7.20	6.95	5.85	6.00

* The initial oxygen content of the solutions was approximately 8.1 p.p.m. at greenhouse temperature.

mum development of the plants. On the other hand, the data show that the oxygen content of the aërated solutions was maintained at a relatively high level, but even in these solutions the action of the plants reduced the oxygen pressure so that it was always slightly below the initial pressure although the solutions were vigorously and continuously aërated.

It has already been pointed out that maintaining the oxygen supply of the nutrient solutions through aëration has no beneficial influence upon the growth of the plant tops and only slightly increases the root yields when grown in the nutrient solutions which were only intermittently renewed. This is in accord with Pember's (5) report that barley did not respond to aëration when grown in solutions which were renewed every two weeks. Free (2) likewise has reported that buckwheat showed no response to aëration when grown in solution cultures with intermittent solution renewal. On the other hand, the data of tables 1 and 3 show that the maintenance, through vigorous aëration, of a high content of dissolved oxygen in the culture solutions which were continuously renewed had a very pronounced effect upon the manner of growth (especially with reference to the roots) and upon the final yields of soybeans. The average total yield from the aërated-drip sand cultures was 26 percent higher than the corresponding yield from the drip sand cultures without aëration. Likewise, the continuously renewed solution cultures with aëration produced an average total yield 49 percent above the corresponding yield from the drip-solution cultures without aëration. As previously stated, the solutions of all these cultures were renewed by a constant drip at the rate of one liter per culture in twenty-four hours. Whether or not a higher rate of solution flow could influence the final yields has not been tested.

H-ion Concentration Relations

An important consideration to which little attention has as yet been given is the relation of the change of hydrogen-ion concentration in culture solutions under various experimental conditions to the development of the plants. While the cause of this well defined reaction change resulting from

contact of plant roots with nutrient solutions of certain definite composition has not as yet been fully explained, it is generally recognized as being largely the result of differential ion-absorption by the plant roots.

In the solutions used in the present experiments the plants were able to change the reaction from an initial pH value of 4.6 to one quite near the neutral point. The data for the sand cultures will not be considered here since they were found to be in quite close agreement with those for the solution cultures. The latter are presented in graphic form in figure 2. Since aëration was found to have no effect upon reaction change, the solid line in figure 2 represents the averages of the pH value of corresponding cultures of the two series with intermittent solution renewal, while the broken line represents the averages of the pH values of corresponding cultures of the two series with continuous solution renewal.

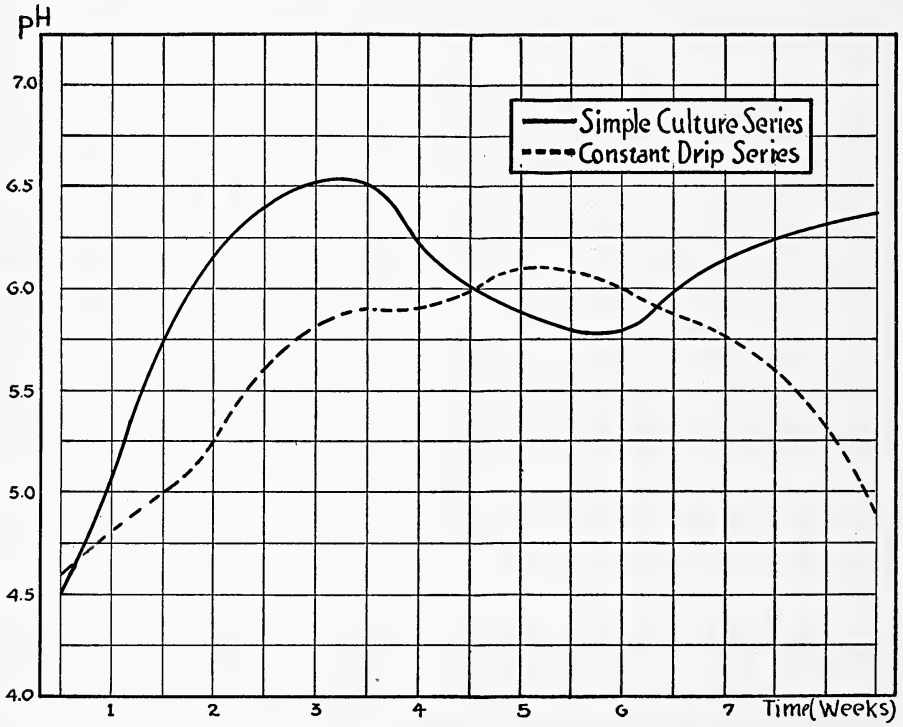

FIG. 2. Graphs of pH values of the culture solutions in contact with the plant roots at intervals during the growth period.

The graph representing the pH values of the cultures in which the solutions were continuously renewed indicates that the capacity of the plants to produce reaction change in the nutrient solutions increases gradually but uniformly to a maximum at five weeks. This was approximately the time when the plants of these cultures reached their highest vegetative activity. Following this, the power of the plants to produce reaction

change diminishes gradually with approaching maturity of the plants, as the graph shows. This graph represents the usual trend of reaction change produced by normal soybean plants in these three-salt solutions. However, it will be observed from a comparison of this graph with that representing the pH values of the solutions of the non-drip cultures that a constant drip which supplied one liter of new solution to a culture during a twenty-four hour period considerably retarded reaction change but is by no means an effective method of controlling this influence of the plants upon the medium.

The graph representing the pH values of the non-drip cultures is markedly divergent from that representing the pH values of the drip cultures, the former having reached a maximum reaction change considerably greater than that of the latter and occurring two weeks earlier. It is quite probable that this pronounced and rapid reaction change in the solutions of the non-drip cultures during the very early stages of growth may result in retarded plant activity during subsequent development. Such retarded activity is actually shown for the plants of the non-drip cultures following the third week of the growth period, if the relative rates of reaction change produced by the plants may be taken as an index. This is clearly indicated by the marked decline in the graph of pH values for these cultures during the fourth, fifth, and sixth weeks. It is to be noted also that the growth of the plants of these cultures was abnormal, showing thick and brittle leaves and very thick and highly pubescent stems, somewhat like the abnormal stem growth of soybean plants described by McCall (4). This was found to be particularly noticeable in the aërated-non-drip cultures. The plants of the non-drip cultures were also considerably later in maturing seed than were those of the drip cultures. Seeds from the drip cultures were tested for germination and were found to germinate well and to produce normal seedlings. Seeds from the non-drip cultures, on the other hand, were neither so well developed nor so mature as were those from the drip cultures.

Summary

This paper presents the results of a preliminary study of aëration and continuous renewal of nutrient culture solution as related to the growth of soybeans in solution and sand cultures. Some of the main points to be emphasized in a tentative way may be summarized as follows:

1. Solution or sand cultures with continuous solution renewal by which one liter of new solution per culture was supplied during each 24-hour interval throughout the growth period always produced plants which were superior in every respect to those grown in cultures with intermittent solution renewal. However, the best plants with respect to health, vigor, and yields always occurred in the cultures with continuous solution renewal and with constant and thorough aëration of the medium.

2. Aëration of solution or sand cultures which maintained the maximum supply of dissolved oxygen (approximate saturation) in the culture solu-

tions renewed intermittently had no apparent influence upon the growth of soybean tops, but it produced considerable increase in root development.

3. Aëration of solution or sand cultures with continuous solution renewal produced a marked increase in the growth of both tops and roots of soybeans.

4. Continuous renewal of the nutrient solution did not alone maintain the supply of dissolved oxygen necessary for maximum development of the plants.

5. The response of roots to aëration was much more pronounced in the cultures in which the solutions were continuously renewed than it was in those in which the solutions were only intermittently renewed.

6. The superiority of roots grown in the aërated media over those grown in the non-aërated was manifested not so much in higher yield weights as in the development of very efficient absorbing systems consisting of long, slender main roots thickly beset with well developed laterals, giving rise to very extensive absorbing surfaces.

7. The superior root growth resulting from aëration of the cultures with continuously renewed solutions was particularly reflected in accelerated top growth during the later stages of development.

8. The average total yields obtained from the sand cultures were throughout uniformly much higher than were those obtained from the solution cultures.

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New Jersey Agricultural Experiment Station

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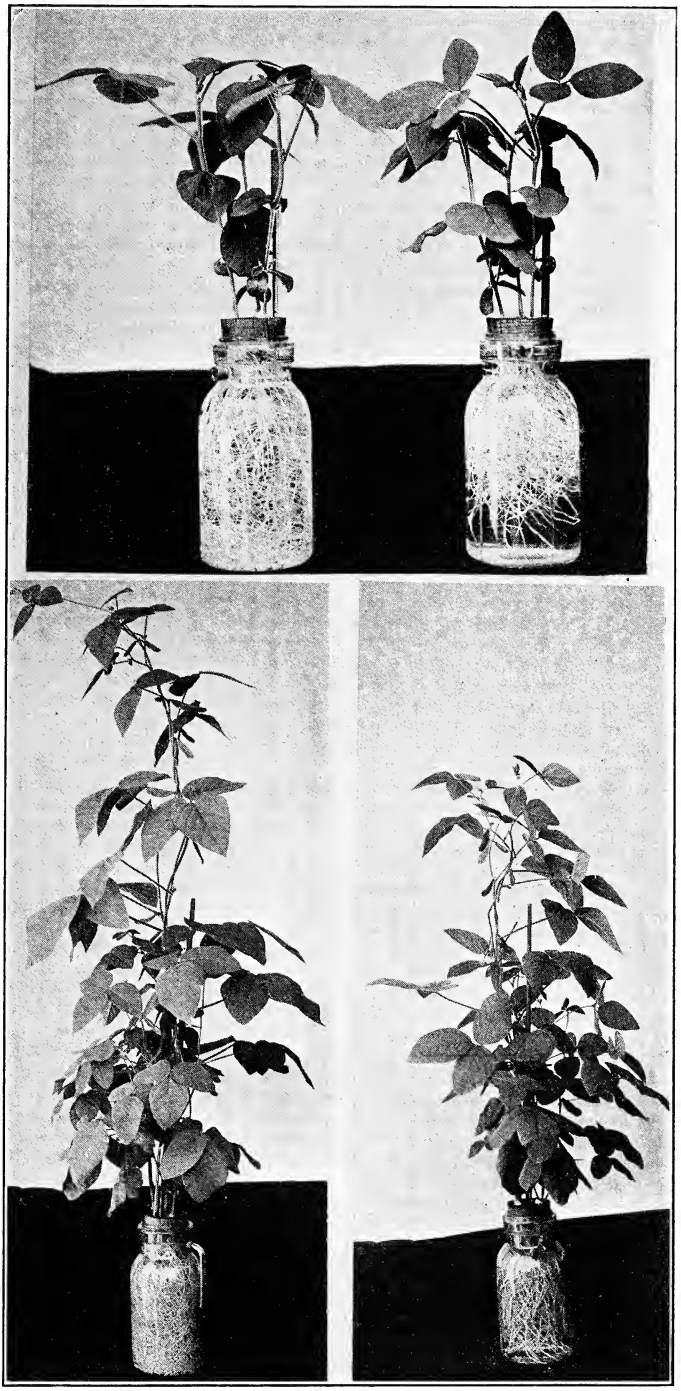
EXPLANATION OF PLATE XXXIX

Above. Drip solution cultures at the age of 14 days. Culture on the left aërated, culture on the right not aërated. Note the superior root development of the culture on the left.

Below. Same cultures as those shown above, at the age of 37 days. Culture on left aërated, culture on right not aërated. Note how completely the roots fill the jar on the left.



ALLISON AND SHIVE: GROWTH OF SOYBEANS

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(New names and final members of new combinations are in **heavy-face** type.)

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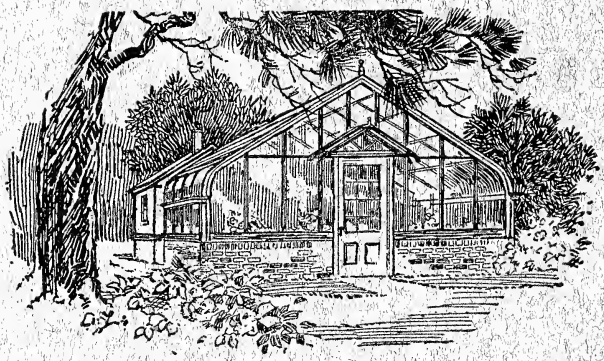

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