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AN ENUMERATION OF THE HERBACEOUS PLANTS COLLECTED BY J. F. ROCK FOR THE ARNOLD ARBORETUM[1]

ALFRED REHDER and CLARENCE E. KOBUSKI

FUNGI (LICHENES)

Determined at the Farlow Herbarium

Cladonia gracilis (L.) Willd. var. **elongata** (Jacq.) Floerke
Southwestern Kansu: Tao River basin, nos. 13187, 14937.
Lobaria pulmonaria (L.) Hoffm. var. **hypomela** Crombie
Southwestern Kansu: Lower Tebbu country, nos. 14848, 14867.
Peltigera aphthosa (L.) Willd.
Southwestern Kansu: Tao River basin, no. 13188.
Sticta Henryana Muell.-Arg.
Southwestern Kansu: Lower Tebbu country, no. 14839.

POLYPODIACEAE

Determined by E. B. COPELAND

Woodsia lanosa Hook.
Southwestern Kansu: Lower Tebbu country, no. 14879.
Woodsia macrospora C. Christensen & Maxon in Jour. Wash. Acad. Sci. XVII. 499 (1927).
Southwestern Kansu: Tao River basin, no. 13712; Lower Tebbu country, no. 14780.
Cystopteris montana (Lam.) Bernh.
Southwestern Kansu: Lower Tebbu country, no. 14871.
Pteretis Struthiopteris (L.) Niewl.
Southwestern Kansu: Lower Tebbu country, no. 14731.

[1]For the enumeration of the ligneous plants collected for the Arnold Arboretum by J. F. Rock see Jour. Arnold Arb. IX. 4-27, 37-125 (1928); XIII. 385-409 (1932).

Except when stated otherwise the plants in the following enumeration have been determined at the Botanic Museum, Berlin-Dahlem.

New species and varieties based on material collected by J. F. Rock are marked with an asterisk (*).

Dryopteris paleacea (Swartz) C. Chr. var. **khasiana** (Clarke) C. Christensen in Contrib. U. S. Nat. Herb. XXVI. 280 (1931).

Dryopteris filix mas (L.) Schott var. *khasiana* Clarke

Southwestern Kansu: Lower Tebbu country, no. 14841.

Dryopteris Robertiana (Hoffm.) C. Chr.

Southwestern Kansu: Lower Tebbu country, no. 14778.

Polystichum Braunii (Spenn.) Fée

Southwestern Kansu: Lower Tebbu country, no. 14840.

Polystichum molliculum Christ

Southwestern Kansu: Tao River basin, no. 13135; Lower Tebbu country, no. 14822. Eastern Tibet: Radja and Yellow River gorges, no. 14027 (in part).

Asplenium varians Wall.

Eastern Tibet: Radja and Yellow River gorges, no. 14027 (in part).

Athyrium acrostichoides (Sw.) Diels

Southwestern Kansu: Lower Tebbu country, nos. 14842, 14869.

Athyrium filix femina (L.) Roth

Southwestern Kansu: Upper Tebbu country, no. 14634; Lower Tebbu country, no. 14697.

Athyrium filix femina (L.) Roth var. **cyclosorum** Rupr.

Southwestern Kansu: Lower Tebbu country, no. 14705.

Athyrium spinulosum (Maxim.) Milde

Southwestern Kansu: Lower Tebbu country, no. 14779.

Notholaena Delavayi (Baker) C. Christensen in Contrib. U. S. Nat. Herb. XXVI. 307 (1931).

Gymnogramme Delavayi Baker

Gymnopteris Delavayi Underw.

Southwestern Kansu: Lower Tebbu country, no. 14881.

Cheilanthes argentea (Gmel.) Kze.

Eastern Tibet: grasslands between Labrang and Yellow River, no. 13910.

Adiantum latedeltoideum (Christ) C. Christensen in Act. Hort. Gothob. I. 94 (1924).

Adiantum monochlamys var. *latedeltoideum* Christ

Southwestern Kansu: Lower Tebbu country, no. 14714.

Adiantum pedatum L.

Southwestern Kansu: Tao River basin, no. 14647; Lower Tebbu country, no. 14870.

Polypodium clathratum C. B. Clarke

Eastern Tibet: Radja and Yellow River gorges, no. 14028. Southwestern Kansu: Lower Tebbu country, no. 14880.

Polypodium eilophyllum Diels in Act. Hort. Gothob. I. 100 (1924).

Southwestern Kansu: Lower Tebbu country, no. 14719.

Polypodium lineare Thunb.
Southwestern Kansu: Lower Tebbu country, no. 14854.
Polypodium lineare Thunb. var.
Southwestern Kansu: Tao River basin, no. 12140.
Cyclophorus taeniodes C. Chr.
Southwestern Kansu: Tao River basin, no. 12085.
Cyclophorus sticticus (Kze.) C. Chr.
Southwestern Kansu: Lower Tebbu country, no. 14715.
Cyclophorus pekinensis C. Chr.
Southwestern Kansu: Tao River basin, no. 12139.

LYCOPODIACEAE

Selaginella spec.
Southwestern Kansu: Tao River basin, no. 12085 A.

EQUISETACEAE

Equisetum arvense L.
Western Szechuan: no. 12040.
Equisetum spec.
Eastern Tibet: Radja and Yellow River gorges, no. 13984.

JUNCAGINACEAE

Triglochin maritimum L.
Southwestern Kansu: Tao River basin, no. 12273. Eastern Tibet: Radja and Yellow River gorges, no. 14009; Ba Valley, no. 14246.

GRAMINEAE

Determined by A. S. Hitchcock

Torresia odorata (L.) Hitchc.
Eastern Tibet: Radja and Yellow River gorges, no. 14107.
Stipa conferta Poir.
Eastern Tibet: Kokonor region, no. 13383.
Stipa splendens Trin.
Eastern Tibet: Kokonor region, no. 13393.
Stipa mongholica Turcz.
Eastern Tibet: grasslands between Labrang and Yellow River, no. 14530.
Aira caespitosa L.
Eastern Tibet: grasslands between Labrang and Yellow River, nos. 14454, 14456, 14528.
Trisetum spicatum Richt.
Eastern Tibet: grasslands between Labrang and Yellow River, nos. 14479, 14488.

Trisetum spec.
Eastern Tibet: grasslands between Labrang and Yellow River, nos. 14457, 14473.
Beckmannia erucaeformis (L.) Host
Central Kansu: no. 12680.
Phragmites communis Trin.
Eastern Tibet: Kokonor region, no. 13387.
Koeleria cristata (L.) Pers.
Eastern Tibet: grasslands between Labrang and Yellow River, no. 14451.
Poa attenuata Trin.
Eastern Tibet: grasslands between Labrang and Yellow River, nos. 14446, 14477, 14480.
Poa bulbosa L.
Southwestern Kansu: Tao River basin, no. 13741.
Poa flexuosa Muhl.
Eastern Tibet: grasslands between Labrang and Yellow River, no. 14452.
Poa sphondylodes Trin.
Southwestern Kansu: Tao River basin, nos. 12562, 13177.
Poa aff. *P. arctica* R. Br.
Eastern Tibet: grasslands between Labrang and Yellow River, no. 14453.
Poa spec.
Eastern Tibet: grasslands between Labrang and Yellow River, no. 14472.
Festuca ovina L.
Eastern Tibet: grasslands between Labrang and Yellow River, no. 14778.
Agropyron longearistatum Boiss.
Southwestern Kansu: Tao River basin, no. 13711.
Elymus sibiricus L.
Eastern Tibet: grasslands between Labrang and Yellow River, no. 14455.

CYPERACEAE

Determined by G. KÜKENTHAL

Eriophorum comosum Nees
Western Szechuan: no. 12023.
***Kobresia kansuensis** Kükenthal in Notizbl. Bot. Gard. Mus. Berlin, x. 882 (1930).
Southwestern Kansu: Tao River basin, no. 13714 (holotype).
Kobresia Prattii C. B. Clarke

Eastern Tibet: grasslands between Labrang and Yellow River, no. 14663.

Carex atrata L. var. **aterrima** (Hoppe) Hartm.

Southwestern Kansu: Tao River basin, no. 13726.

Carex atrata L. ssp. **pullata** (Boott) Kükenth.

Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14162.

Carex atrofusca Schkuhr

Southwestern Kansu: Tao River basin, no. 12311.

ARACEAE

Arisaema consanguineum Schott f. **latisectum** Engl.

Southwestern Kansu: Tao River basin, no. 12431.

JUNCACEAE

Juncus leucomelas Royle

Central Kansu: no. 12715.

Juncus Thomsoni Buchen.

Eastern Tibet: Ba Valley, no. 14248.

LILIACEAE

Hemerocallis Dumortieri Morr.

Central Kansu: no. 12770.

Gagea pauciflora Turcz.

Southwestern Kansu: Tao River basin, no. 12093.

Allium chrysanthum Regel

Southwestern Kansu: Tao River basin, nos. 13704, 14622.

Allium cyaneum Regel

Western Kansu: nos. 13252, 13253. Southwestern Kansu: Tao River basin, no. 13722.

Allium Henryi C. H. Wright

Southwestern Kansu: Upper Tebbu country, no. 13048.

Allium kansuense Regel

Southwestern Kansu: Tao River basin, no. 13728; Lower Tebbu country, nos. 14885, 14887.

Allium monadelphum Turcz. var. **thibeticum** Regel

Southwestern Kansu: Tao River basin, no. 12401. Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14389.

Allium polyrhizum Turcz.

Eastern Tibet: Ba Valley, no. 14364.

Allium Przewalskianum Regel

Southwestern Kansu: Tao River basin, no. 12588.

Allium tanguticum Regel
Southwestern Kansu: Tao River basin, no. 12903.
Allium victorialis L.
Southwestern Kansu: Tao River basin, no. 12847.
Allium spec.
Southwestern Kansu: Tao River basin, nos. 12616, 12852; Upper Tebbu country: no. 13037. Central Kansu: no. 12789. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14449; alpine region between Radja and Jupar ranges, nos. 14095, 14389.
Lilium Davidi Duch.
Southwestern Kansu: Tao River basin, no. 12592 A.
Lilium Duchartrei Franch.
Central Kansu: no. 13618.
Lilium Duchartrei Franch. var. **Farreri** Krause
Southwestern Kansu: Tao River basin, no. 13145; Upper Tebbu country, no. 14608. Central Kansu: nos. 12666, 12668.
Lilium tenuifolium Fisch.
Southwestern Kansu: Tao River basin, nos. 12589, 12592, 13606.
Fritillaria cirrhosa Don var. **ecirrhosa** Franch.
Southwestern Kansu: Tao River basin, 12197, 12481; Upper Tebbu country, no. 13085.
Fritillaria Roylei Hook.
Eastern Tibet: Radja and Yellow River gorges, no. 14102; alpine region between Radja and Jupar ranges, nos. 14160, 14225.
Lloydia tibetica Bak. var. **lutescens** Franch.
Southwestern Kansu: Tao River basin, no. 12600.
Lloydia tibetica Bak. var. **purpurascens** Franch.
Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14143.
Asparagus brachyphyllus Turcz.
Southwestern Kansu: Tao River basin, nos. 12172, 12915.
Asparagus trichophyllus Bge.
Southwestern Kansu: Tao River basin, no. 12176.
Clintonia udensis Trautv. & Mey.
Southwestern Kansu: Lower Tebbu country, no. 14812.
Polygonatum bulbosum Lévl.
Eastern Tibet: Radja and Yellow River gorges, nos. 13987, 14082.
Polygonatum sibiricum Ledeb.
Southwestern Kansu: Tao River basin, nos. 12561, 12639.
Polygonatum spec.
Southwestern Kansu: Tao River basin, nos. 12241, 12315. Eastern Tibet: Radja and Yellow River gorges, no. 14106.

Paris polyphylla Sm.
Southwestern Kansu: Tao River basin, no. 12224.

DIOSCOREACEAE

Dioscorea quinqueloba Thunb.
Southwestern Kansu: Lower Tebbu country, no. 14752.

IRIDACEAE

Iris dichotoma Pall.
Southwestern Kansu: Lower Tebbu country, no. 14746.
Iris ensata Thunb.
Southwestern Kansu: Tao River basin, nos. 12111, 12323; Upper Tebbu country, no. 13637.
Iris gracilis Maxim.
Southwestern Kansu: Tao River basin, no. 12340. Eastern Tibet: Radja and Yellow River gorges, nos. 13983, 14078.
Iris Potanini Maxim.
Eastern Tibet: Radja and Yellow River gorges, nos. 13945, 13964, 13975; Jupar Range, no. 14338.
Iris tenuifolia Pall.
Southwestern Kansu: Tao River basin, nos. 12100, 12101. Eastern Tibet: Radja and Yellow River gorges, no. 13950.
Iris spec.
Southwestern Kansu: Tao River basin, nos. 12138, 12851.

ORCHIDACEAE

Determined by C. SCHWEINFURTH

Cypripedium luteum Franch.
Southwestern Kansu: Upper Tebbu country, no. 12536.
Cypripedium nutans Schlecht.
Southwestern Kansu: Upper Tebbu country, no. 12499.
Cypripedium tibeticum King.
Southwestern Kansu: Upper Tebbu country, no. 12521. Eastern Tibet: Radja and Yellow River gorges, nos. 13989, 14165.
Aceratorchis tschiliensis Schlechter
Eastern Tibet: Radja and Yellow River gorges, no. 14190.
Orchis chusua D. Don.
Central Kansu: no. 12735. Southwestern Kansu: Tao River basin, no. 12833; Upper Tebbu country, no. 13115.
Orchis salina Turcz.
Eastern Tibet: Radja and Yellow River gorges, no. 13978; Ba Valley, nos. 14251, 14356.
Orchis spathulata Reichb.

Central Kansu: no. 12713.

Amitostigma monanthum (Finet) Schlechter

Southwestern Kansu: Tao River basin, no. 12622.

Herminium tanguticum Rolfe

Central Kansu: no. 12736.

Habenaria conopsea Benth.

Southwestern Kansu: Tao River basin, no. 12944; Upper Tebbu country, no. 13120.

Habenaria cucullata (L.) Hoefft

Southwestern Kansu: Lower Tebbu country, no. 14700.

Habenaria spiranthiformis Ames & Schlechter

Eastern Tibet: Radja and Yellow River gorges, no. 14210.

***Amnesia longibracteata** Schweinfurth in Jour. Arnold Arb. x. 172 (1929).

Central Kansu: no. 13216 (holotype).

***Oreorchis Rockii** Schweinfurth in Jour. Arnold Arb. x. 173 (1929).

Central Kansu: no. 12744 (holotype).

MORACEAE

Humulus lupulus L.

Southwestern Kansu: Tao River basin, nos. 13207, 13716; Lower Tebbu country, no. 14753.

URTICACEAE

Urtica dioica L.

Eastern Tibet: Radja and Yellow River gorges, no. 14024.

POLYGONACEAE

Rheum acuminatum Hook. f. & Thoms.

Central Kansu: no. 12673.

Rheum palmatum L.

Eastern Tibet: Radja and Yellow River gorges, nos. 14071, 14167; Jupar Range, no. 14313.

Rheum palmatum L. floribus rubris

Southwestern Kansu: Tao River basin, no. 12844. Eastern Tibet: Radja and Yellow River gorges, no. 13982.

Rheum pumilum Maxim.

Southwestern Kansu: Tao River basin, no. 12409b. Eastern Tibet: Radja and Yellow River gorges, nos. 14038, 14173.

Rheum spiciforme Royle

Eastern Tibet: Jupar Range, no. 14330.

Polygonum Hookeri Meisn.

Southwestern Kansu: Tao River basin, nos. 12409a, 12604. East-

ern Tibet: alpine region between Radja and Jupar ranges, nos. 14221, 14376.

Polygonum sphaerostachyum Meisn. var.

Eastern Tibet: Jupar Range, no. 14335.

CHENOPODIACEAE

Eurotia ceratoides C. A. Mey.

Eastern Tibet: grasslands between Labrang and Yellow River, no. 14545.

CARYOPHYLLACEAE

Arenaria kansuensis Maxim.

Southwestern Kansu: Upper Tebbu country, no. 13043. Eastern Tibet: alpine region between Radja and Jupar ranges, nos. 14149, 14222.

Arenaria melanandra (Maxim.) Mattfeld apud Handel-Mazzetti, Symb. Sin. VII. 202 (1929).

Eastern Tibet: alpine region between Radja and Jupar ranges, nos. 14157, 14379.

Arenaria Przewalskii Maxim.

Southwestern Kansu: Upper Tebbu country, no. 13082. Eastern Tibet: Amnyi Machen range, no. 14431; grasslands between Labrang and Yellow River, no. 14508.

Arenaria spec.

Southwestern Kansu: Upper Tebbu country, no. 13083.

Silene Fortunei Vis.

Southwestern Kansu: Lower Tebbu country, no. 14745.

Silene repens Patrin

Southwestern Kansu: Tao River basin, no. 12865.

Silene tenuis Willd.

Eastern Tibet: Radja and Yellow River gorges, no. 14209.

Melandryum apetalum (L.) Fenzl

Eastern Tibet: grasslands between Labrang and Yellow River, nos. 14476, 14500.

Melandryum apetalum (L.) Fenzl forma

Central Kansu: no. 12737.

Melandryum glandulosum F. N. Williams

Eastern Tibet: Radja and Yellow River gorges, no. 14121.

Gypsophila Gmelini Bge.

Central Kansu: no. 13236.

Dianthus chinensis L.

Southwestern Kansu: Tao River basin, nos. 13209, 14650. Central Kansu: no. 12752.

Dianthus superbus L.

Central Kansu: no. 12686. Southwestern Kansu: Tao River basin, nos. 12892, 13155. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14540.

RANUNCULACEAE

Paeonia anomala L.

Southwestern Kansu: Tao River basin, nos. 12260, 12276, 12456, 13593.

Paeonia Veitchii Lynch

Southwestern Kansu: Tao River basin, nos. 12235, 13127.

Caltha palustris L.

Southwestern Kansu: Tao River basin, no. 12229.

*__Caltha scaposa__ Hook. f. & Thoms. var. **Smithii** Ulbrich in Notizbl. Bot. Gard. Mus. Berlin, x. 864 (1929).

Southwestern Kansu: Tao River basin, no. 12317 (syntype).

Trollius pumilus D. Don

Southwestern Kansu: Tao River basin, nos. 12354, 12606, 14623; Upper Tebbu country, no. 13002. Central Kansu: no. 12697. Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14220.

*__Trollius pumilus__ D. Don var. **alpinus** Ulbrich in Notizbl. Bot. Gard. Mus. Berlin, x. 865 (1929).

Eastern Tibet: Radja and Yellow River gorges, nos. 13976, 13988, 14050 (syntypes).

Helleborus thibetanus Franch.

Southern Kansu: no. 12059.

Paraquilegia anemonoides (Willd.) Ulbr.

Southwestern Kansu: Tao River basin, nos. 12393, 12629, 12812; Upper Tebbu country, nos. 12494, 12514. Eastern Tibet: Radja and Yellow River gorges, nos. 13973, 14067, 14068, 14069.

*__Urophysa Rockii__ Ulbrich, n. gen. & sp., in Notizbl. Bot. Gard. Mus. Berlin, x. 869 (1929).

Western Szechuan: no. 12015 (holotype).

Souliea vaginata (Maxim.) Franch.

Southwestern Kansu: Tao River basin, nos. 12304, 12412.

Actaea spicata L. var. **erythrocarpa** Ledeb.

Southwestern Kansu: Lower Tebbu country, no. 14849.

Aquilegia ecalcarata Maxim.

Southwestern Kansu: Tao River basin, nos. 12206, 12439, 12945.

Aquilegia oxysepala Trautv. & Mey.

Southwestern Kansu: Tao River basin, no. 12887.

Delphinium albocoeruleum Maxim.

Southwestern Kansu: Tao River basin, no. 13194. Eastern Tibet: grasslands between Labrang and Yellow River, nos. 14459, 14467, 14501, 14542, 14543.

Delphinium coelestinum Franch.

Southwestern Kansu: Tao River basin, no. 14625.

Delphinium densiflorum Duthie

Eastern Tibet: grasslands between Labrang and Yellow River, no. 14493.

Delphinium Forrestii Diels

Southwestern Kansu: Tao River basin, no. 13740. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14535.

Delphinium grandiflorum L.

Southwestern Kansu: Tao River basin, no. 13178; Upper Tebbu country, no. 14594.

Delphinium Henryi Franch.

Southwestern Kansu: Tao River basin, nos. 13154, 13193. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14547.

*__Delphinium labrangense__ Ulbrich, spec. nov. ined.

Eastern Tibet: grasslands between Labrang and Yellow River, nos. 14482, 14483, 14504, 14513.

Delphinium Maximowiczii Franch.

Southwestern Kansu: Upper Tebbu country, nos. 13086, 13093, 14644.

Delphinium Pylzowi Maxim.

Southwestern Kansu: Upper Tebbu country, no. 13024; Tao River basin, no. 13185.

Delphinium Souliei Franch.

Southwestern Kansu: Upper Tebbu country, no. 13092. Eastern Tibet: Kokonor region, no. 13398; grasslands between Labrang and Yellow River, nos. 14460, 14502, 14518, 14523.

Delphinium sparsiflorum Maxim.

Southwestern Kansu: Tao River basin, no. 13191.

Delphinium tanguticum Huth

Southwestern Kansu: Upper Tebbu country, nos. 13023 (in part), 13041, 13088; Tao River basin, no. 14641.

Delphinium tatsienense Franch.

Eastern Tibet: Kokonor region, no. 13361; grasslands between Labrang and Yellow River, no. 14538. Southwestern Kansu: Tao River basin, nos. 13136, 13730.

Delphinium tongolense Franch.

Southwestern Kansu: Tao River basin, no. 13161; Upper Tebbu country, nos. 13109, 13111.

Aconitum Anthora L.

Eastern Tibet: grasslands between Labrang and Yellow River, no. 14494. Southwestern Kansu: Lower Tebbu country, no. 14677.

Aconitum Anthora L. var. **anthoroideum** (Rehb.) Regel

Southwestern Kansu: Tao River basin, nos. 13182, 13710, 13722, 13735, 14620; Upper Tebbu country, no. 13000. Eastern Tibet: Kokonor region, no. 13363.

Aconitum gymnandrum Maxim.

Southwestern Kansu: Tao River basin, nos. 12183, 12242, 12285, 12351, 12877.

Aconitum laeve Royle

Central Kansu: no. 12739. Southwestern Kansu: Upper Tebbu country, no. 13079; Tao River basin, 13162. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14541.

Aconitum Napellus L. var. **semigaleatum** Pall.

Southwestern Kansu: Tao River basin, no. 13721.

Aconitum rotundifolium K. & K. var. **tanguticum** Maxim.

Southwestern Kansu: Tao River basin, nos. 14621, 14643; Upper Tebbu country, nos. 13023 (in part), 13039, 13050. Central Kansu: no. 12694. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14515; Amnyi Machen range, no. 14440.

Aconitum volubile Pall.

Southwestern Kansu: Tao River basin, nos. 13205, 13743; Lower Tebbu country, no. 14692.

Aconitum volubile Pall. var. **flexuosum** (Rchb.) Rap.

Southwestern Kansu: Tao River basin, nos. 14615, 14860.

Aconitum volubile Pall. var.

Southwestern Kansu: Tao River basin, no. 14649.

Anemone demissa Hook. f. & Thoms.

Southwestern Kansu: Tao River basin, nos. 12596, 12838.

Anemone imbricata Maxim.

Eastern Tibet: Radja and Yellow River gorges, nos. 14049, 14146; alpine region between Radja and Jupar ranges, no. 14231; Jupar Range, no. 14371; Amnyi Machen range, no. 14421.

Anemone japonica (Thunb.) S. & Z. var. **tomentosa** Maxim.

Central Kansu: no. 12766.

Anemone narcissiflora L.

Southwestern Kansu: Upper Tebbu country, no. 13625.

Anemone rivularis Buch.-Ham.

Southwestern Kansu: Tao River basin, no. 12357. Eastern Tibet: Radja and Yellow River gorges, no. 14128.

***Anemone Rockii** Ulbrich in Notizbl. Bot. Gard. Mus. Berlin, x. 877 (1929).

Southwestern Kansu: Tao River basin, nos. 12408a, 12487 (syntypes); Upper Tebbu country, nos. 12520, 13061, 13626 (syntypes).

Anemone rupestris Wall.

Southwestern Kansu: Tao River basin, no. 12254. Eastern Tibet: Radja and Yellow River gorges, nos. 13992, 14017, 14103, 14105, 14196.

Anemone vitifolia Buch.-Ham. var. **tomentosa** Maxim.

Southwestern Kansu: Tao River basin, no. 13213; Upper Tebbu country, no. 14609.

Pulsatilla ambigua Turcz.

Southwestern Kansu: Tao River basin, no. 12182.

Pulsatilla spec.

Eastern Tibet: Radja and Yellow River gorges, no. 14048.

Oxygraphis glacialis Bge.

Southwestern Kansu: Tao River basin, no. 12390. Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14099.

Ranunculus acris L.

Southwestern Kansu: Tao River basin, no. 12566.

Ranunculus affinis R. Br.

Southwestern Kansu: Tao River basin, no. 12280. Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14405.

Ranunculus affinis R. Br. var. **flabellatus** Franch.

Southwestern Kansu: Tao River basin, no. 12160.

Ranunculus affinis R. Br. var. **tangutica** Maxim.

Southwestern Kansu: Tao River basin, no. 12446.

Ranunculus Flammula L.

Southwestern Kansu: Tao River basin, no. 12282.

Ranunculus japonicus Thunb.

Southwestern Kansu: Tao River basin, no. 12908.

Ranunculus pulchellus C. A. Mey.

Southwestern Kansu: Tao River basin, no. 12281. Eastern Tibet: Radja and Yellow River gorges, no. 13991.

Ranunculus pulchellus C. A. Mey. var. **sericeus** Hook. f. & Thoms.

Eastern Tibet: Radja and Yellow River gorges, no. 14197.

Ranunculus yunnanensis Franch.

Southwestern Kansu: Tao River basin, no. 12342.

Thalictrum alpinum L.

Southwestern Kansu: Tao River basin, no. 12248.

Thalictrum baicalense Turcz.

Southwestern Kansu: Tao River basin, no. 12862.

Thalictrum javanicum Bl.

Southwestern Kansu: Tao River basin, no. 12835. Eastern Tibet: Ba Valley, no. 14271.

Thalictrum Przewalskii Maxim.

Southwestern Kansu: Upper Tebbu country, no. 13054.

BERBERIDACEAE

Podophyllum emodi Wall.

Southwestern Kansu: Tao River basin, nos. 12259, 12350, 12424, 14901.

Epimedium brevicornu Maxim.

Western Szechuan: no. 12016.

Epimedium pubescens Maxim.

Southwestern Kansu: Tao River basin, no. 12302.

Leontice robusta B. Fedtsch.

Southwestern Kansu: Lower Tebbu country, no. 14716.

PAPAVERACEAE

Hypecoum erectum L. var. **lactiflorum** (Kar. & Kir.) Maxim.

Southwestern Kansu: Tao River basin, no. 12272.

Dicranostigma spec.

Southwestern Kansu: Tao River basin, no. 13144.

Macleaya microcarpa (Maxim.) Fedde

Southwestern Kansu: Lower Tebbu country, nos. 14655, 14690.

Bocconia spec.

Southwestern Kansu: Lower Tebbu country, no. 15095.

Meconopsis integrifolia Franch.

Southwestern Kansu: Tao River basin, nos. 12416, 13651; Upper Tebbu country, no. 13073. Eastern Tibet: Radja and Yellow River gorges, nos. 13996, 14086.

Meconopsis psilonomma Farrer

Southwestern Kansu: Tao River basin, no. 12613; Upper Tebbu country, no. 13632.

Meconopsis punicea Maxim.

Southwestern Kansu: Tao River basin, nos. 12195, 12423, 12840, 12942; Upper Tebbu country, nos. 13053, 13642. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14506.

Meconopsis quintuplinervia Regel

Southwestern Kansu: Tao River basin, nos. 12360, 12608, 12611, 13620; Upper Tebbu country, no. 13046. Central Kansu: 12700, 12742. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14462; alpine region between Radja and Jupar ranges, nos. 14144, 14224; Jupar Range, no. 14309.

Meconopsis racemosa Maxim.

Southwestern Kansu: Tao River basin, nos. 12621, 12625, 13160; Upper Tebbu country, nos. 12537, 13009, 13627. Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14377; Jupar Range, no. 14339; Amnyi Machen range, no. 14434.

Meconopsis spec.

Southwestern Kansu: Upper Tebbu country, no. 13077. Eastern Tibet: Amnyi Machen range, no. 14430.

Corydalis adunca Maxim.

Eastern Tibet: Radja and Yellow River gorges, no. 14046.

Corydalis curviflora Maxim.

Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14092.

Corydalis curviflora Maxim. var. **cytisiflora** Fedde

Southwestern Kansu: Upper Tebbu country, no. 12503.

Corydalis curviflora Maxim. var. **pseudo-Smithii** Fedde

Southwestern Kansu: Tao River basin, no. 12576.

Corydalis curviflora Maxim. var. **Smithii** Fedde

Southwestern Kansu: Tao River basin, no. 12414.

Corydalis dasyptera Maxim.

Southwestern Kansu: Tao River basin, nos. 12387, 12602; Upper Tebbu country, no. 13007. Eastern Tibet: Radja and Yellow River gorges, no. 14189; alpine region between Radja and Jupar ranges, no. 14237; Jupar Range, nos. 14311, 14312, 14336.

Corydalis glycyphyllos Fedde

Eastern Tibet: alpine region between Radja and Jupar ranges, nos. 14374, 14375.

Corydalis linarioides Maxim.

Southwestern Kansu: Upper Tebbu country, no. 13018.

Corydalis melanochlora Maxim.

Southwestern Kansu: Upper Tebbu country, no. 13021. Eastern Tibet: Jupar Range, no. 14329.

Corydalis Rheinbabeniana Fedde

Southwestern Kansu: Upper Tebbu country, no. 13003. Eastern Tibet: Amnyi Machen range, no. 14419.

Corydalis scaberula Maxim.

Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14226.

Corydalis straminea Maxim.

Southwestern Kansu: Tao River basin, nos. 12178, 12462, 13138.

Corydalis stricta Steph.

Eastern Tibet: Radja and Yellow River gorges, nos. 14019, 14114, 14184.

Corydalis trachycarpa Maxim.
Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14155.
Corydalis spec.
Eastern Tibet: Radja and Yellow River gorges, no. 14047.

CRUCIFERAE

Megacarpaea Delavayi Franch.
Southwestern Kansu: Upper Tebbu country, no. 13087.
*__Megacarpaea Delavayi__ Franchet var. **grandiflora** O. E. Schulz in Notizbl. Bot. Gard. Mus. Berlin, x. 557 (1929).
Southwestern Kansu: Tao River basin, no. 12597 (holotype).
Dilophia fontana Maxim.
Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14398.
Dilophia macrosperma O. E. Schulz
Eastern Tibet: Amnyi Machen range, no. 14414.
Cochlearia scapiflora Hook. f. & Thoms.
Eastern Tibet: alpine region between Radja and Jupar ranges, nos. 14236, 14396.
Eutrema compactum O. E. Schulz
Eastern Tibet: Jupar Range, no. 14340.
Eruca sativa Lam. var. **lativalvis** (Boiss.) Coss.
Southwestern Kansu: Tao River basin, no. 12179.
Brassica juncea (L.) Coss.
Eastern Tibet: Radja and Yellow River gorges, no. 14180.
Cardamine macrophylla Willd.
Eastern Tibet: Radja and Yellow River gorges, nos. 14025, 14066.
Cardamine macrophylla Willd. ssp. **polyphylla** (Don) O. E. Schulz in Notizbl. Bot. Gard. Mus. Berlin, ix. 1071 (1927).
Southwestern Kansu: Upper Tebbu country, no. 12508.
Cardamine tangutorum O. E. Schulz
Southwestern Kansu: Tao River basin, no. 12318.
*__Draba lanceolata__ Royle var. **latifolia** O. E. Schulz in Notizbl. Bot. Gard. Mus. Berlin, x. 555 (1929).
Eastern Tibet: Radja and Yellow River gorges, no. 14134 (holotype).
Draba lanceolata Royle var. **leiocarpa** O. E. Schulz in Notizbl Bot. Gard. Mus. Berlin, ix. 1077 (1929).
Southwestern Kansu: Upper Tebbu country, no. 12491. Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14223; Radja and Yellow River gorges, no. 14130.
Draba lichiangensis W. W. Sm.

Eastern Tibet: Jupar Range, no. 14369.

Draba oreades Schrenk proles **chinensis** O. E. Schulz in Engler, Pflanzenr. IV.-105 (Heft 89) 109 (1927).

Southwestern Kansu: Tao River basin, no. 12388.

Draba oreades Schrenk var. **commutata** (Reg.) O. E. Schulz in Engler, Pflanzenr. IV.-105 (Heft 89) 109 (1927).

Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14228.

Draba oreades Schrenk var. **racemosa** O. E. Schulz in Engler, Pflanzenr. IV.-105 (Heft 89) 109 (1927).

Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14091.

Draba oreades Schrenk var. **Tafelii** O. E. Schulz in Engler, Pflanzenr. IV.-105 (Heft 89) 108 (1927).

Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14142.

*__Draba Rockii__ O. E. Schulz in Notizbl. Bot. Gard. Mus. Berlin, x. 555 (1929).

Southwestern Kansu: Tao River basin, no. 12405 (holotype).

Arabis alaschanica Maxim.

Southwestern Kansu: Upper Tebbu country, nos. 12490, 12515.

Cheiranthus roseus Maxim.

Eastern Tibet: Jupar Range, no. 14409.

*__Cheiranthus roseus__ Maxim. f. **caespitosus** O. E. Schulz, msc.

Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14096.

*__Cheiranthus roseus__ Maxim. f. **elatior** O. E. Schulz, msc.

Eastern Tibet: Jupar Range, no. 14345.

Malcolmia africana (L.) R. Br.

Eastern Tibet: Radja and Yellow River gorges, no. 14133.

Torularia humilis (C. A. Mey.) O. E. Schulz

Southwestern Kansu: Tao River basin, no. 12243. Eastern Tibet: Radja and Yellow River gorges, no. 14131.

Torularia humilis (C. A. Mey.) O. E. Schulz prol. **Piasezkii** (Maxim.) O. E. Schulz

Southwestern Kansu: Tao River basin, no. 12269.

Torularia humilis f. **grandiflora** O. E. Schulz

Southwestern Kansu: Tao River basin, no. 12171.

Dontostemon glandulosus (Kar. & Kir.) O. E. Schulz in Notizbl. Bot. Gard. Mus. Berlin, x. 554 (1929).

Arabis glandulosa Kar. & Kir.

Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14399.

Parrya villosa Maxim.
Eastern Tibet: Radja and Yellow River gorges, nos. 14051, 14088.
*__Parrya villosa__ Maxim. var. __albiflora__ O. E. Schulz in Notizbl. Bot. Gard. Mus. Berlin, x. 557 (1929).
Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14392 (holotype).

CRASSULACEAE

Sedum Aizoon L.
Southwestern Kansu: Tao River basin, no. 12914.
Sedum Aizoon L. var. **scabrum** Maxim.
Southwestern Kansu: Tao River basin, no. 12355.
Sedum algidum Ledeb.
Southwestern Kansu: Tao River basin, no. 14153.
Sedum algidum Ledeb. var. **tanguticum** Maxim.
Eastern Tibet: Jupar Range, nos. 14346, 14365.
Sedum Henryi Diels forma **gracilis** (♂)
Southwestern Kansu: Tao River basin, no. 12839.
Sedum Kirilowi Regel
Eastern Tibet: Radja and Yellow River gorges, no. 14174.
*__Sedum progressum__ Diels in Notizbl. Bot. Gard. Mus. Berlin, x. 887 (1930).
Southwestern Kansu: Tao River basin, no. 12849 (holotype).
Sedum Purdomii W. W. Sm.
Central Kansu: no. 12705.
Sedum quadrifidum Pall.
Eastern Tibet: Radja and Yellow River gorges, no. 13981.
Sedum venustum Praeger
Southwestern Kansu: Upper Tebbu country, no. 12516. Eastern Tibet: Radja and Yellow River gorges, no. 14042.
Sedum spec.
Western Kansu: no. 13259.

SAXIFRAGACEAE

Saxifraga confertifolia Engl. & Irmsch.
Southwestern Kansu: Upper Tebbu country, no. 13027.
Saxifraga diversifolia Wall. var. **Soulieana** Engl. & Irmsch.
Eastern Tibet: Kokonor region, no. 13356. Southwestern Kansu: Upper Tebbu country, no. 14585.
Saxifraga diversifolia Wall. var.
Southwestern Kansu: Tao River basin, no. 14642.
Saxifraga flagrans H. Smith var. **platyphylla** H. Smith.
Southwestern Kansu: Tao River basin, nos. 12196, 12817.

Saxifraga Giraldiana Engl.
Southwestern Kansu: Upper Tebbu country, no. 13051.
*__Saxifraga kansuensis__ Mattfeld in Notizbl. Bot. Gard. Mus. Berlin, x. 887 (1930).
Southwestern Kansu: Tao River basin, no. 12624 (syntype); Upper Tebbu country, no. 12525 (syntype).
Saxifraga lumpuensis Engl.
Central Kansu: no. 12743. Southwestern Kansu: Tao River basin, no. 12832.
Saxifraga melanocentra Franch. f. **Franchetiana** Engl. & Irmsch.
Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14158.
Saxifraga melanocentra Franch. var. **pluriflora** Engl. & Irmsch.
Eastern Tibet: Amnyi Machen range, no. 14441.
Saxifraga melanocentra Franch. var.
Southwestern Kansu: Upper Tebbu country, no. 13042.
Saxifraga montana H. Smith var. **splendens** H. Smith
Southwestern Kansu: Tao River basin, no. 14613.
Saxifraga Przewalskii Engl.
Southwestern Kansu: Upper Tebbu country, no. 13017. Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14232; Jupar Range, no. 14327.
Saxifraga pseudo-hirculus Engl.
Southwestern Kansu: Upper Tebbu country, no. 13089. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14527.
Saxifraga tangutica Engl.
Southwestern Kansu: Upper Tebbu country, no. 12510. Eastern Tibet: Radja and Yellow River gorges, no. 14169.
Saxifraga unguiculata Engl.
Eastern Tibet: grasslands between Labrang and Yellow River, no. 14514.
Tiarella polyphylla D. Don
Central Kansu: no. 12679.
Chrysosplenium nudicaule Bge.
Eastern Tibet: Jupar Range, no. 14334.
Chrysosplenium sphaerospermum Maxim.
Southwestern Kansu: Tao River basin, no. 12097.
Parnassia Delavayi Franch.
Central Kansu: no. 12790.
Parnassia setchuenensis Franch.
Southwestern Kansu: Tao River basin, no. 13157; Upper Tebbu country, no. 14589.

ROSACEAE

Aruncus sylvester Kostel.
Southwestern Kansu: Tao River basin, no. 12815.
Fragaria elatior Ehrb.
Eastern Tibet: Radja and Yellow River gorges, no. 14036.
Potentilla Anserina L.
Southwestern Kansu: Tao River basin, no. 12180. Eastern Tibet: Radja and Yellow River gorges, no. 14022; grasslands between Labrang and Yellow River, no. 14532.
Potentilla biflora Willd.
Southwestern Kansu: Upper Tebbu country, no. 13006.
Potentilla bifurca L.
Southwestern Kansu: Tao River basin, no. 12223. Eastern Tibet: Kokonor region, no. 13351.
Potentilla multicaulis Bge.
Eastern Tibet: Radja and Yellow River gorges, no. 14176.
Potentilla Potaninii Th. Wolf
Southwestern Kansu: Tao River basin, no. 12859.
Potentilla Salesoviana Steph.
Northwestern Kansu: no. 13321. Eastern Tibet: Ba Valley, no. 14280.
Potentilla Saundersiana Royle
Eastern Tibet: alpine region between Radja and Jupar ranges, nos. 14150, 14378, 14391.
Potentilla sericea L.
Southwestern Kansu: Tao River basin, no. 12857.
Potentilla spec.
Southwestern Kansu: Tao River basin, no. 12911. Eastern Tibet: Radja and Yellow River gorges, no. 14175; grasslands between Labrang and Yellow River, no. 14486.
Coluria longifolia Maxim.
Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14141.
Sanguisorba canadensis L.
Central Kansu: no. 12777. Southwestern Kansu: Tao River basin, no. 13204.
Sanguisorba officinalis L.
Central Kansu: no. 12778.

LEGUMINOSAE

Sophora alopecuroides L.
Northwestern Kansu: no. 13317.

Thermopsis alpina Ledeb.
Southwestern Kansu: Tao River basin, no. 12155. Eastern Tibet: Radja and Yellow River gorges, no. 14055.
Thermopsis lanceolata R. Br.
Southwestern Kansu: Tao River basin, no. 12338. Eastern Tibet: Radja and Yellow River gorges, no. 13919.
Medicago ruthenica Trautv.
Southwestern Kansu: Tao River basin, no. 12924.
Gueldenstaedtia diversifolia Maxim.
Eastern Tibet: Radja and Yellow River gorges, no. 14123.
Astragalus melilotoides Pall.
Southwestern Kansu: Tao River basin, nos. 12896, 12901.
Astragalus monadelphus Bge.
Southwestern Kansu: Tao River basin, no. 12958.
Astragalus skythropus Bge.
Eastern Tibet: alpine region between Radja and Jupar ranges, nos. 14151, 14394; Jupar Range, no. 14315.
Astragalus tanguticus Batal.
Eastern Tibet: Radja and Yellow River gorges, no. 14182; Ba Valley, no. 14269.
Astragalus spec. aff. *A. subumbellatus* Klotzsch
Eastern Tibet: Radja and Yellow River gorges, no. 14004; Ba Valley, no. 14253.
Astragalus spec.
Southwestern Kansu: Tao River basin, nos. 12084, 12092, 12098, 12220, 12221, 12284, 12327, 12447, 12628, 12802, 12925, 12926, 12953, 13146; Upper Tebbu country, no. 13045. Central Kansu: nos. 12704, 12718. Western Kansu: no. 13254. Eastern Tibet: Kokonor region, nos. 13375, 13382; grasslands between Labrang and Yellow River, no. 14490; alpine region between Radja and Jupar ranges, nos. 14148, 14163, 14233, 14243, 14381; Ba Valley, nos. 14249, 14250, 14254, 14256, 14257, 14258, 14286, 14361; Jupar Range, nos. 14343, 14370.
Oxytropis spec.
Southwestern Kansu: Upper Tebbu country, no. 13065.
Hedysarum esculentum Ledeb.
Southwestern Kansu: Tao River basin, no. 13142; Upper Tebbu country, no. 14607.
Hedysarum obscurum L.
Eastern Tibet: Radja and Yellow River gorges, no. 14171; alpine region between Radja and Jupar ranges, no. 14382.
Hedysarum pseudastragalus Ulbrich

Eastern Tibet: Amnyi Machen range, no. 14423.

Hedysarum spec.

Southwestern Kansu: Tao River basin, no. 12623; Upper Tebbu country, nos. 13010, 13063. Central Kansu: no. 12675. Eastern Tibet: Jupar Range, no. 14347.

Vicia amoena Ledeb. var. **elliptica** Freyn

Southwestern Kansu: Tao River basin, no. 12922.

Vicia cracca L.

Southwestern Kansu: Tao River basin, no. 12959.

Vicia unijuga A. Br.

Southwestern Kansu: Tao River basin, no. 12583.

Vicia unijuga A. Br. var.

Southwestern Kansu: Upper Tebbu country, no. 13064.

Lathyrus pratensis L.

Southwestern Kansu: Tao River basin, no. 12585.

GERANIACEAE

Geranium eriostemon Fisch.

Southwestern Kansu: Tao River basin, nos. 12225, 12575, 12920.

Geranium Pylzowianum Maxim.

Southwestern Kansu: Tao River basin, nos. 12450, 12582, 12825, 12918. Eastern Tibet: Radja and Yellow River gorges, no. 14212.

Geranium spec. aff. *G. pratense* L.

Southwestern Kansu: Tao River basin, no. 12855. Eastern Tibet: Radja and Yellow River gorges, no. 14216; Ba Valley, no. 14360.

Erodium Stephanianum Willd.

Eastern Tibet: Radja and Yellow River gorges, no. 14205.

Biebersteinia heterostemon Maxim.

Southwestern Kansu: Tao River basin, no. 12904.

LINACEAE

Linum nutans Maxim.

Southwestern Kansu: Tao River basin, no. 12170.

ZYGOPHYLLACEAE

Peganum Harmala L.

Central Kansu: no. 13229. Eastern Tibet: Jupar Range, no. 14317.

Zygophyllum mucronatum Maxim.

Central Kansu: no. 13241.

Tribulus terrestris L.

Northwestern Kansu: no. 13397.

POLYGALACEAE

Polygala sibirica L.

Eastern Tibet: Radja and Yellow River gorges, no. 14204.

EUPHORBIACEAE

Euphorbia spec.
Eastern Tibet: Radja and Yellow River gorges, no. 13941.

GUTTIFERAE

Hypericum Przewalskii Maxim.
Southwestern Kansu: Tao River basin, no. 12875, 12909. Eastern Tibet: Radja and Yellow River gorges, no. 14213.
Hypericum spec.
Central Kansu: no. 12772.

TAMARICACEAE

Hololachne songarica Ehrenb.
Central Kansu: no. 13235.

VIOLACEAE

Viola biflora L.
Southwestern Kansu: Upper Tebbu country, no. 12493. Eastern Tibet: Radja and Yellow River gorges, nos. 14015, 14084.
Viola bulbosa Maxim.
Eastern Tibet: Radja and Yellow River gorges, no. 14029.
Viola mongolica Franch. var. flor. carneis
Eastern Tibet: Radja and Yellow River gorges, no. 13966.

THYMELAEACEAE

Stellera Chamaejasme L.
Southwestern Kansu: Tao River basin, nos. 12238, 12286. Eastern Tibet: Radja and Yellow River gorges, no. 14020.
Stellera Chamaejasme L. fl. albo
Eastern Tibet: Radja and Yellow River gorges, no. 14199.

OENOTHERACEAE

Epilobium angustifolium L.
Central Kansu: no. 12755. Southwestern Kansu: Tao River basin, no. 12954.

UMBELLIFERAE

Determined by C. NORMAN

Bupleurum longeradiatum Turcz.
Central Kansu: no. 12769.
Bupleurum spec.[1]
Eastern Tibet: Radja and Yellow River gorges, no. 14211.

[1]Perhaps this specimen represents *B. microcephalum* Diels. However, the specimen at hand is without true leaves, hence it cannot be determined with certainty.

Pleurospermum Candollii C. B. Clarke[1]

Central Kansu: no. 12703.

Pleurospermum cnidiifolium Wolff in Act. Hort. Gothob. II. 292 (1926).

Eastern Tibet: Kokonor region, no. 13359.

***Pleurospermum Dielsianum** Fedde & Wolff in Fedde, Rep. Spec. Nov. XXVII. 121 (1929).

Eastern Tibet: grasslands between Labrang and Yellow River, no. 14466 (syntype).

Pleurospermum Franchetianum Hemsley in Hooker, Icon. XXIII. 2244 (1894).

Pleurospermum Rockii* Fedde & Wolff in Fedde, Rep. Spec. Nov. XXVII. 120 (1929).—Synon. nov.**

Pleurospermum Pilgerianum* Fedde & Wolff in Fedde, Rep. Spec. Nov. XXVII. 121 (1929).—Synon. nov.**

Southwestern Kansu: Tao River basin, no. 12227 (holotype of *P. Rockii* Fedde & Wolff), no. 12614 (holotype of *P. Pilgerianum* Fedde & Wolff).

Pleurospermum linearilobum W. W. Smith in Notes Bot. Gard. Edinb. VIII. 342 (1915).[2]

**Pleurospermum Dielsianum* Fedde & Wolff in Fedde, Rep. Spec. Nov. XXVII. 121 (1929), as to no. 14178.

Eastern Tibet: Radja and Yellow River gorges, no. 14178 (syntype of *P. Dielsianum* Fedde & Wolff).

***Pleurospermum pseudo-involucratum** Wolff in Fedde, Rep. Spec. Nov. XXVII. 119 (1929).[3]

Southwestern Kansu: Upper Tebbu country, no. 12496 (holotype).

Pleurospermum thalictrifolium Wolff in Act. Hort. Gothob. II. 297 (1926).

Eastern Tibet: Jupar Range, no. 14372; Amnyi Machen range, no. 14432.

Pleurospermum spec. aff. *P. Candollii* C. B. Clarke

Eastern Tibet: Jupar Range, no. 14307 (incomplete material).

Carum carvi L.

[1]This determination is given with some hesitation as the specimen for study is very young. However, it agrees in all essentials with the other material examined. The involucels are shorter than those of the Indian specimens, which may be due to the immature condition of the specimens. It is a widely dispersed species having been recorded from Kashmir, Kumaon, Tian shan and Nepal. It is doubtful if it has been recorded before as far east as Kansu.

[2]The specimen no. 14178 is cited in the original description of *P. Dielsianum* Fedde & Wolff as identical with no. 14466. No. 14466 is rather fragmentary and is referred here to *P. Dielsianum;* it does not seem to be the same plant as no. 14178, which is undoubtedly *P. linearilobum* W. W. Sm.

[3]Wolff was in doubt whether this specimen should be described under *Pleurospermum* or *Ligusticum*, but the doubt lies rather between *Pleurospermum* and *Trachydium*. The final decision will depend on more ample material.

Southwestern Kansu: Tao River basin, no. 12449. Eastern Tibet: Radja and Yellow River gorges, no. 14117; Ba Valley, no. 14255.

Tongoloa elata Wolff in Act. Hort. Gothob. II. 291 (1925).

Southwestern Kansu: Upper Tebbu country, no. 14629.

Ligusticum Pilgerianum Wolff in Fedde, Rep. Spec. Nov. XXVII. 307 (1930).

Central Kansu: no. 12724.

Ligusticum sinense Oliver in Hooker, Icon. XX. 1958 (1891).

**Ligusticum Pilgerianum* Fedde in Fedde, Rep. Spec. Nov. XXVII. 322 (1930), non Wolff (p. 307).

Southwestern Kansu: Upper Tebbu country, no. 14590 (syntype of *L. Pilgerianum* Fedde).

***Ligusticum Weberbauerianum** Fedde & Wolff in Fedde, Rep. Spec. Nov. XXVII. 312 (1930).

Central Kansu: no. 12727 (holotype).

Heracleum millefolium Diels

Southwestern Kansu: Tao River basin, no. 12222. Eastern Tibet: Radja and Yellow River gorges, no. 14207; grasslands between Labrang and Yellow River, no. 14491.

***Heracleum millefolium** Diels var. **longilobum** Norman, var. nov.[1]

A typo differt foliorum segmentis ultimis longioribus acutioribusque, et ovarii indumento densiore.

Central Kansu. Lien hoa shan: among rocks between Tao Chow and Titao, alt. 3450 m., no. 12734 (type), July 1925 (plant white, woolly; flowers carmine); among rocks between Tao-chow and Titao, alt. 3050 m., no. 12674, July 1925 (plant rosette-like; flowers pinkish lavender).

PYROLACEAE

Pyrola rotundifolia L.

Central Kansu: no. 12745.

PRIMULACEAE

Primula L.

Determined by W. W. SMITH

Primula aerinantha Balf. f. & Purdom

Central Kansu: nos. 12676, 12711.

Primula alsophila Balf. f. & Farrer

Southwestern Kansu: Tao River basin, no. 12819.

Primula chionantha Balf. f. & Forrest

Southwestern Kansu: Tao River basin, no. 12392; Upper Tebbu country, no. 13631.

[1]Dr. Diels, to whom part of this specimen was submitted, intimated that he considered it "at least a variety or an allied species."

Primula chionantha Balf. f. & Forrest (farinose form)
Southwestern Kansu: Tao River basin, nos. 12345, 12375.
Primula chionantha Balf. f. & Forrest var.
Southwestern Kansu: Tao River basin, no. 12470.
Primula chionantha Balf. f. & Forrest forma
Southwestern Kansu: Tao River basin, nos. 13656, 14920.
Primula conspersa Balf. f. & Purdom
Southwestern Kansu: Tao River basin, nos. 12451 (in part), 12563, 12381, 12874. Central Kansu: no. 12683.
Primula flava Maxim.
Southwestern Kansu: Tao River basin, no. 12363; Upper Tebbu country, nos. 12492, 13639. Eastern Tibet: Radja and Yellow River gorges, nos. 13965, 14043, 14120; grasslands between Labrang and Yellow River, no. 13914.
Primula gemmifera Batal.
Southwestern Kansu: Tao River basin, nos. 12451 (in part), 12818, 13159, 14624; Upper Tebbu country, nos. 13004, 13029. Eastern Tibet: Radja and Yellow River gorges, no. 13993.
Primula graminifolia Pax & Hoffm.
Southwestern Kansu: Tao River basin, nos. 12384, 12404; Upper Tebbu country, no. 13014.
Primula limbata Balf. f. & Forrest
Southwestern Kansu: Tao River basin, nos. 12381, 12408, 13654; Upper Tebbu country, no. 13066. Eastern Tibet: Radja and Yellow River gorges, no. 14064.
Primula longipetiolata Pax & Hoffm. vel aff.
Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14227.
Primula moupinensis Franch.
Southern Kansu: nos. 12043, 12069.
Primula optata Farrer apud I. B. Balf.
Southwestern Kansu: Upper Tebbu country, no. 13633. Eastern Tibet: alpine region between Radja and Jupar ranges, nos. 14097, 14145, 14147, 14385.
Primula optata Farrer apud I. B. Balf. forma
Eastern Tibet: Amnyi Machen range, no. 14424.
Primula polyneura Franch.
Southwestern Kansu: Tao River basin, nos. 12251, 12316, 12438, 13652.
Primula pumilio Maxim.
Southwestern Kansu: Tao River basin, no. 12406.
Primula Purdomii Craib

Southwestern Kansu: Tao River basin, nos. 12346, 12383, 12403. Eastern Tibet: Radja and Yellow River gorges, no. 14089.

Primula reginella Balf. f.

Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14238.

Primula reginella Balf. f. (dwarf form)

Eastern Tibet: grasslands between Labrang and Yellow River, no. 13907.

Primula sibirica Jacq.

Southwestern Kansu: Tao River basin, nos. 12277, 12313. Eastern Tibet: Radja and Yellow River gorges, no. 13994; Ba Valley, no. 14276.

Primula sikkimensis Hook. f.

Eastern Tibet: Radja and Yellow River gorges, no. 14166; Amnyi Machen range, no. 14437.

Primula stenocalyx Maxim.

Southwestern Kansu: Tao River basin, nos. 12091, 12109, 12307, 12421, 12488; Upper Tebbu country, no. 12523. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14470.

Primula stenocalyx Maxim. (farinose form)

Southwestern Kansu: Tao River basin, nos. 12361, 12402. Central Kansu: no. 12733.

Primula tangutica Duthie

Southwestern Kansu: Tao River basin, nos. 12253, 12261, 12612, 13619, 13655; Upper Tebbu country, no. 12512. Eastern Tibet: Radja and Yellow River gorges, nos. 14008, 14065; Jupar Range, no. 14310.

Primula Woodwardii Balf. f.

Southwestern Kansu: Tao River basin, nos. 12653, 12656, 12837, 13658. Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14234.

Primula spec.

Southwestern Kansu: Tao River basin, no. 12407.

Androsace L.

Determined by H. HANDEL-MAZZETTI

Androsace Mariae Kan. var. **tibetica** (Maxim.) Handel-Mazzetti in Act. Hort. Gothob. II. 114 (1926).

Southwestern Kansu: Tao River basin, nos. 12094, 12133, 12308, 12619. Eastern Tibet: Radja and Yellow River gorges, nos. 13902, 13940, 14083; Ba Valley, no. 14261.

Androsace tapete Maxim.

Southwestern Kansu: Tao River basin, no. 12650. Central Kansu: no. 12741.

Androsace yargongensis Petitmengin

Southwestern Kansu: Upper Tebbu country, no. 13040.

Cortusa Matthioli L.

Central Kansu: no. 12720.

Glaux maritima L.

Southwestern Kansu: Tao River basin, no. 12245. Eastern Tibet: Radja and Yellow River gorges, no. 14135.

PLUMBAGINACEAE

Statice bicolor Bge.

Central Kansu: no. 12758.

GENTIANACEAE

Gentiana L.

Determined by C. V. B. MARQUAND

Gentiana algida Pall. forma

Eastern Tibet: grasslands between Labrang and Yellow River, no. 14510.

Gentiana dahurica Fisch.

Eastern Tibet: Kokonor region, no. 13368; grasslands between Labrang and Yellow River, no. 14531.

Gentiana Farreri Balf. f.

Southwestern Kansu: Tao River basin, no. 14886; Lower Tebbu country, no. 14862.

Gentiana Futtereri Diels & Gilg

Eastern Tibet: Kokonor region, nos. 13360, 13399.

Gentiana gracilipes Turrill

Southwestern Kansu: Tao River basin, nos. 13175, 13719.

Gentiana hexaphylla Maxim.

Southwestern Kansu: Tao River basin, no. 13713.

Gentiana hexaphylla Maxim. var. **caudata** Marquand in Kew Bull. Misc. Inform. 1931, p. 81.

Southwestern Kansu: Tao River basin, no. 13736.

Gentiana leucomelaena Maxim.

Southwestern Kansu: Tao River basin, no. 12309. Eastern Tibet: Ba Valley, no. 14277.

Gentiana Przewalskii Maxim.

Southwestern Kansu: Tao River basin, nos. 13720, 13734, 14645; Upper Tebbu country, no. 13011.

Gentiana quinquenervia Turrill

Southwestern Kansu: Tao River basin, no. 13179.

Gentiana riparia Kar. & Kir.
Eastern Tibet: Radja and Yellow River gorges, no. 14021.
Gentiana siphonantha Maxim.
Eastern Tibet: Kokonor region, no. 13364; grasslands between Labrang and Yellow River, no. 14499 (partly browsed).
Gentiana spathulifolia Kusnez.
Southwestern Kansu: Upper Tebbu country, no. 14606.
Gentiana straminea Maxim.
Eastern Tibet: Kokonor region, no. 13380.
Gentiana straminea Maxim. forma (leaves wider than the type).
Southwestern Kansu: Tao River basin, no. 13715.
Gentiana striata Maxim.
Southwestern Kansu: Lower Tebbu country, no. 14863.
Gentiana Szechenyii Kanitz (forma plicis longioribus).
Southwestern Kansu: Lower Tebbu country, no. 14861.
Gentiana tetraphylla Kusnez.
Southwestern Kansu: Tao River basin, no. 14638.
Gentianella (§ Crossopetalum) spec.
Eastern Tibet: Radja and Yellow River gorges, no. 14218; Ba Valley, no. 14247. Southwestern Kansu: Tao River basin, no. 12353; Upper Tebbu country, nos. 13031, 14597; Tao River basin, no. 13709. Central Kansu: no. 12691.
Gentianella spec.
Eastern Tibet: Kokonor region, nos. 13347, 13388; grasslands between Labrang and Yellow River, no. 14497.
Swertia spec.
Eastern Tibet: Kokonor region, no. 13350, 13358. Southwestern Kansu: Tao River basin, nos. 13707, 13708, 14619.

ASCLEPIADACEAE

Cynanchum inamoenum (Maxim.) Loes.
Southwestern Kansu: Tao River basin, no. 12226. Central Kansu: no. 13230.

CONVOLVULACEAE

Convolvulus Ammannii Desr.
Central Kansu: no. 13233. Eastern Tibet: Ba Valley, no. 14358.
Convolvulus arvensis L. var. **sagittifolius** Fisch.
Eastern Tibet: Radja and Yellow River gorges, no. 14200; Ba Valley, no. 14359.

POLEMONIACEAE

Polemonium coeruleum L. ssp. **vulgare** (Ledeb.) Brand
Southwestern Kansu: Tao River basin, nos. 12574, 12848, 12919.

BORAGINACEAE

Determined by I. M. JOHNSTON

Lappula Redowskii (Horn.) Greene

Southwestern Kansu: Tao River basin, no. 12358.

Eritrichium strictum Dcne.

Eastern Tibet: Radja and Yellow River gorges, no. 14203.

Lithospermum officinale L.

Southwestern Kansu: Tao River basin, no. 12356.

***Microula Rockii** Johnston in Contrib. Gray Herb. LXXXI. 82 (1928).

Anoplocaryum Rockii (Johnst.) Brand in Fedde, Rep. Spec. Nov. XXVI. 170 (1929).

Southwestern Kansu: Tao River basin, no. 12605 (paratype). Eastern Tibet: grasslands between Labrang and Yellow River, no. 14511 (holotype); alpine region between Radja and Jupar ranges, no. 14384 (paratype).

Microula sikkimensis (Clarke) Hemsley

Southwestern Kansu: Tao River basin, nos. 12250, 12347. Eastern Tibet: Radja and Yellow River gorges, no. 14026; Ba Valley, no. 14275.

Microula tangutica Maxim.

Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14397; grasslands between Labrang and Yellow River, nos. 14469, 14509.

Myosotis alpestris F. W. Schmidt var.

Southwestern Kansu: Tao River basin, nos. 12386, 13005.

LABIATAE

Ajuga calantha Diels

Southwestern Kansu: Tao River basin, nos. 12214, 12455, 12610, 12636, 12806.

***Ajuga calantha** Diels f. **albiflora** Diels, msc.

Central Kansu: no. 12787.

Ajuga lupulina Maxim.

Southwestern Kansu: Tao River basin, no. 12321. Eastern Tibet: Radja and Yellow River gorges, no. 14129; Jupar Range, no. 14373.

Scutellaria amoena C. H. Wright

Southwestern Kansu: Tao River basin, nos. 12564, 13198.

***Scutellaria scordiifolia** Fisch. f. **pubescens** Diels, msc.

Southwestern Kansu: Tao River basin, no. 12900.

***Scutellaria Rehderiana** Diels in Notizbl. Bot. Gard. Mus. Berlin, x. 889 (1930).

Southwestern Kansu: Tao River basin, no. 12827 (holotype).

Marrubium incisum Benth.

Eastern Tibet: Radja and Yellow River gorges, no. 14194.

Nepeta coerulescens Maxim. f. **major** Diels, msc.

Eastern Tibet: Radja and Yellow River gorges, no. 14119.

Nepeta macrantha Fisch.

Southwestern Kansu: Tao River basin, nos. 12891, 12905, 12963; Upper Tebbu country, no. 14628.

Dracocephalum heterophyllum Benth.

Southwestern Kansu: Tao River basin, no. 12894. Central Kansu: nos. 12768, 13247. Western Kansu: no. 13251. Eastern Tibet: Kokonor region, no. 13353; Radja and Yellow River gorges, no. 14198.

Dracocephalum imberbe Bge.

Southwestern Kansu: Tao River basin, nos. 12593, 12871. Central Kansu: no. 12767.

Dracocephalum tanguticum Maxim.

Southwestern Kansu: Tao River basin, nos. 12860, 12866. Western Kansu: nos. 13249, 13260.

Phlomis rotata Benth.

Eastern Tibet: Radja and Yellow River gorges, no. 14172; alpine region between Radja and Jupar ranges, no. 14406.

Phlomis umbrosa Turcz.

Central Kansu: no. 12791. Southwestern Kansu: Upper Tebbu country, no. 14576.

Galeopsis Tetrahit L.

Southwestern Kansu: Tao River basin, no. 13153.

Stachys baicalensis Fisch.

Central Kansu: no. 12665. Eastern Tibet: Radja and Yellow River gorges, no. 14181.

Salvia Prattii Hemsl.

Eastern Tibet: Amnyi Machen range, no. 14426.

Salvia Przewalskii Maxim.

Southwestern Kansu: Tao River basin, no. 12579.

Salvia Roborowskii Maxim.

Southwestern Kansu: Tao River basin, no. 12184.

*__Thymus Serpyllum__ L. ssp. **mongolicus** Ronniger in Notizbl. Bot. Gard. Mus. Berlin, x. 890 (1930).

Southwestern Kansu: Tao River basin, no. 12927 (syntype). Central Kansu: no. 12664. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14539 (syntype).

Elsholtzia densa Benth.

Southwestern Kansu: Tao River basin, no. 13150.

SOLANACEAE

Anisodus spec.
Southwestern Kansu: Tao River basin, no. 12337.
Solanum septemlobum Bge.
Eastern Tibet: Ba Valley, no. 14273.

SCROPHULARIACEAE

Scrophularia incisa Weinm.
Eastern Tibet: Radja and Yellow River gorges, no. 14137; Ba Valley, no. 14245.
Scrofella chinensis Maxim.
Southwestern Kansu: Tao River basin, nos. 13152, 13733. Central Kansu: no. 12690.
Mimulus nepalensis Benth.
Southwestern Kansu: Tao River basin, no. 12947.
Veronica ciliata Fisch.
Southwestern Kansu: Upper Tebbu country, no. 14600.
Lagotis brachystachya Maxim.
Southwestern Kansu: Tao River basin, no. 12130. Eastern Tibet: Radja and Yellow River gorges, no. 13951.
Lagotis brevituba Maxim.
Eastern Tibet: Jupar Range, no. 14332.
Lagotis glauca Gaertn.
Southwestern Kansu: Tao River basin, no. 12391. Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14098.
Euphrasia hirtella Jord.
Southwestern Kansu: Tao River basin, no. 12246.
Euphrasia tatarica Fisch.
Central Kansu: no. 12788.
Pedicularis alaschanica Maxim.
Southwestern Kansu: Tao River basin, nos. 12459, 12568. Eastern Tibet: Kokonor region, no. 13374.
Pedicularis anas Maxim. var.
Southwestern Kansu: Upper Tebbu country, no. 13035.
Pedicularis armata Maxim.
Southwestern Kansu: Tao River basin, nos. 12880, 13148; Upper Tebbu country, no. 13091.
Pedicularis cheilanthifolia Schrenk
Southwestern Kansu: Tao River basin, no. 12312. Eastern Tibet: grasslands between Labrang and Yellow River, nos. 14465, 14526; Ba Valley, no. 14272.
Pedicularis cheilanthifolia Schrenk var. **isochila** Maxim.

Southwestern Kansu: Tao River basin, nos. 12247, 12278. Eastern Tibet: Radja and Yellow River gorges, no. 14202.

*__Pedicularis chenocephala__ Diels in Notizbl. Bot. Gard. Mus. Berlin, x. 892 (1930).

Southwestern Kansu: Tao River basin, no. 12607 (holotype).

__Pedicularis chenocephala__ Diels forma

Eastern Tibet: Amnyi Machen range, no. 14435.

__Pedicularis chinensis__ Maxim.

Southwestern Kansu: Upper Tebbu country, no. 13101. Eastern Tibet: Kokonor region, no. 13355.

__Pedicularis cranolopha__ Maxim.

Eastern Tibet: Radja and Yellow River gorges, no. 14186.

__Pedicularis cranolopha__ Maxim. var. __longicornuta__ Prain

Southwestern Kansu: Tao River basin, no. 12928.

__Pedicularis cristata__ Maxim.

Southwestern Kansu: Tao River basin, nos. 12828, 12879, 12910.

__Pedicularis Davidi__ Franch.

Southwestern Kansu: Tao River basin, nos. 12448, 12906; Upper Tebbu country, no. 13032.

*__Pedicularis decorissima__ Diels in Notizbl. Bot. Gard. Mus. Berlin, x. 891 (1930).

Eastern Tibet: grasslands between Labrang and Yellow River, nos. 14546 (holotype), 14537 (paratype).

__Pedicularis ingens__ Maxim.

Eastern Tibet: Radja and Yellow River gorges, no. 14217; grasslands between Labrang and Yellow River, no. 14481.

__Pedicularis kansuensis__ Maxim.

Southwestern Kansu: Tao River basin, nos. 12287, 12794. Eastern Tibet: Radja and Yellow River gorges, nos. 14118, 14208; Ba Valley, no. 14270.

__Pedicularis labellata__ Jacq.

Southwestern Kansu: Upper Tebbu country, no. 13098; Tao River basin, no. 13164. Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14403.

__Pedicularis lasiophrys__ Maxim.

Eastern Tibet: Amnyi Machen range, no. 14439; grasslands between Labrang and Yellow River, no. 14525.

__Pedicularis lasiophrys__ Maxim. var. __sinica__ Maxim.

Southwestern Kansu: Tao River basin, no. 12850.

__Pedicularis longiflora__ Rudolphi

Eastern Tibet: Ba Valey, no. 14354.

__Pedicularis macrosiphon__ Franch.

Southwestern Kansu: Tao River basin, no. 12293.

Pedicularis muscicola Maxim.
Eastern Tibet: Ba Valley, no. 14279.
Pedicularis pilostachya Maxim.
Eastern Tibet: Jupar Range, no. 14331.
Pedicularis Przewalskii Maxim.
Eastern Tibet: Amnyi Machen range, no. 14438.
Pedicularis recurva Maxim.
Southwestern Kansu: Upper Tebbu country, no. 13097.
Pedicularis rudis Maxim.
Southwestern Kansu: Tao River basin, no. 12868; Upper Tebbu country, no. 13104.
Pedicularis scolopax Maxim. flor. majoribus
Eastern Tibet: Radja and Yellow River gorges, no. 14132.
Pedicularis semitorta Maxim.
Southwestern Kansu: Tao River basin, nos. 12268, 12795. Central Kansu: no. 12696. Eastern Tibet: Radja and Yellow River gorges, nos. 14127, 14215; grasslands between Labrang and Yellow River, no. 14548.
*__Pedicularis striata__ Pall. var. __poliocalyx__ Diels in Notizbl. Bot. Gard. Mus. Berlin, x. 892 (1930).
Southwestern Kansu: Tao River basin, nos. 12798 (holotype), 12916 (paratype).
Pedicularis szetschuanica Maxim.
Southwestern Kansu: Tao River basin, nos. 12244, 12620. Eastern Tibet: Radja and Yellow River gorges, no. 14191; alpine region between Radja and Jupar ranges, no. 14161; Ba Valley, nos. 14252, 14274; Amnyi Machen range, no. 14416; grasslands between Labrang and Yellow River, no. 14503.
Pedicularis szetschuanica Maxim. var. **longispica** Bon.
Southwestern Kansu: Tao River basin, no. 12643. Eastern Tibet: Radja and Yellow River gorges, no. 14192; Amnyi Machen range, no. 14415.
Pedicularis torta Maxim.
Southwestern Kansu: Tao River basin, nos. 12615, 12923.
Pedicularis tristis L. var. **macrantha** Maxim.
Southwestern Kansu: Tao River basin, no. 12637. Central Kansu: no. 12702. Eastern Tibet: Amnyi Machen range, no. 14422.
Pedicularis versicolor Wahlenb.
Southwestern Kansu: Tao River basin, nos. 12413, 12601, 12642; Upper Tebbu country, no. 13124. Eastern Tibet: Radja and Yellow River gorges, no. 13990; alpine region between Radja and Jupar ranges, nos. 14094, 14235, 14395.

Pedicularis spec. aff. *P. plicata* Maxim.
Central Kansu: no. 12784.
Pedicularis spec.
Southwestern Kansu: Tao River basin, no. 12255.
Pedicularis (Bidentatae) spec.
Southwestern Kansu: Upper Tebbu country, no. 12529.
Pedicularis (Longirostres) spec.
Central Kansu: no. 12689.

BIGNONIACEAE

Incarvillea compacta Maxim.
Southwestern Kansu: Tao River basin, no. 13653. Northwestern Kansu: no. 13301. Eastern Tibet: Radja and Yellow River gorges, nos. 13974, 14087.
Incarvillea principis Bur. & Franch.
Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14401.
Incarvillea sinensis Lam.
Central Kansu: no. 13245.

OROBANCHACEAE

Xylanche himalaica Beck
Southwestern Kansu: Upper Tebbu country, no. 13123.

LENTIBULARIACEAE

Pinguicula alpina L.
Southwestern Kansu: Upper Tebbu country, no. 12535; Tao River basin, no. 12644.

RUBIACEAE

Asperula odorata L.
Southwestern Kansu: Tao River basin, no. 12570.
Galium boreale L.
Southwestern Kansu: Tao River basin, no. 12559.
Galium verum L.
Southwestern Kansu: Tao River basin, no. 12591.
Galium spec.
Southwestern Kansu: Tao River basin, no. 12580. Eastern Tibet: Radja and Yellow River gorges, no. 14195.

CAPRIFOLIACEAE

Triosteum pinnatifidum Maxim.
Southwestern Kansu: Tao River basin, no. 12216; Upper Tebbu country, no. 12509.

VALERIANACEAE

Valeriana pseudodioica Pax & K. Hoffm.
Southwestern Kansu: Tao River basin, no. 12218.
Valeriana tangutica Batal.
Central Kansu: nos. 12712, 12716. Eastern Tibet: Kokonor region, no. 13348; Radja and Yellow River gorges, nos. 13980, 14122.
Valeriana spec.
Southwestern Kansu: Upper Tebbu country, no. 14595.
Nardostachys Jatamansi DC.
Eastern Tibet: Radja and Yellow River gorges, no. 14168; grasslands between Labrang and Yellow River, no. 14484.

DIPSACACEAE

Morina betonicoides Benth.
Southwestern Kansu: Tao River basin, nos. 12617, 14611; Upper Tebbu country, no. 13119. Eastern Tibet: Radja and Yellow River gorges, no. 14170.
Morina chinensis (Batal.) Diels apud Limpricht in Fedde, Rep. Spec. Nov. Beih. XII. 497 (Bot. Reise Hochgeb. Chinas & Ost-Tibets) (1922).
Southwestern Kansu: Tao River basin, nos. 12556, 12952. Eastern Tibet: Radja and Yellow River gorges, no. 14187.

CAMPANULACEAE

Adenophora liliifolioides Pax & K. Hoffm.
Southwestern Kansu: Tao River basin, no. 13151.
Adenophora Potanini Korsh.
Southwestern Kansu: Tao River basin, no. 13140. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14544.
Adenophora Smithii Nannfeldt in Act. Hort. Gothob. v. 21 (1929).
Southwestern Kansu: Tao River basin, no. 12929. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14551.
Adenophora spec. aff. *A. gracilis* Nannfeldt in Act. Hort. Gothob. v. 17 (1929).
Southwestern Kansu: Tao River basin, no. 13149.
Adenophora spec. aff. *A. marsupiiflora* Fisch.
Central Kansu: no. 12688.
Adenophora spec. aff. *A. Potanini* Korsh.
Western Kansu: no. 13250.
Codonopsis ovata Benth.
Eastern Tibet: grasslands between Labrang and Yellow River, no. 14522.
Codonopsis viridiflora Maxim.

Central Kansu: no. 12738. Southwestern Kansu: Upper Tebbu country, no. 13125.

Cyananthus Hookeri C. B. Clarke var. **hispida** Franch.

Southwestern Kansu: Tao River basin, no. 13725.

COMPOSITAE

Aster Bowerii Hemsl.

Eastern Tibet: Kokonor region, no. 13352; grasslands between Labrang and Yellow River, no. 14487.

Aster flaccidus Bge.

Southwestern Kansu: Upper Tebbu country, no. 13008, 13019. Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14230.

Aster Fordii Hemsl.

Southwestern Kansu: Upper Tebbu country, no. 14627

Aster Heterochaeta Benth.

Southwestern Kansu: Tao River basin, nos. 12202, 12252, 12489, 12830. Eastern Tibet: Radja and Yellow River gorges, nos. 13985, 14109.

Aster hispidus Thunb.

Southwestern Kansu: Tao River basin, no. 13706. Western Kansu: no. 13255.

Aster tongolensis Franch.

Southwestern Kansu: Tao River basin, no. 12842. Eastern Tibet: Ba Valley, no. 14285.

Aster trinervius Roxb.

Southwestern Kansu: Tao River basin, no. 13668; Upper Tebbu country, no. 14631.

Aster Vilmorini Franch.

Southwestern Kansu: Tao River basin, nos. 12801, 12950, 14612; Upper Tebbu country, no. 13099. Central Kansu: no. 12684. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14533.

Aster spec.

Southwestern Kansu: Tao River basin, no. 12569. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14447.

Erigeron acris L.

Southwestern Kansu: Upper Tebbu country, no. 14601. Central Kansu: no. 12779.

Leontopodium R. Br.

Determined by H. HANDEL-MAZZETTI

Leontopodium calocephalum (Franch.) Beauvd.

Southwestern Kansu: Upper Tebbu country, no. 13033.

Leontopodium Dedekensii (Bur. & Franch.) Beauvd.
Southwestern Kansu: Tao River basin, no. 13147. Eastern Tibet: Radja and Yellow River gorges, no. 14206.

Leontopodium haplophylloides Hand.-Mazz.
Western Kansu: nos. 13390, 13394. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14550.

Leontopodium Jacotianum Beauvd.
Central Kansu: no. 12714.

Leontopodium linearifolium Hand.-Mazz.
Southwestern Kansu: Upper Tebbu country, no. 13016; Lower Tebbu country, no. 14726. Eastern Tibet: Kokonor region, nos. 13373, 13392; grasslands between Labrang and Yellow River, nos. 14445, 14448, 14461, 14475, 14489, 14492, 14534; alpine region between Radja and Jupar ranges, nos. 14152, 14239, 14242; Jupar Range, no. 14333; Amnyi Machen range, no. 14417.

Leontopodium linearifolium Hand.-Mazz. ad *L. Soulei* Beauvd.
Eastern Tibet: Amnyi Machen range, no. 14418.

Leontopodium nanum (Hook. f. & Thoms.) Handel-Mazzetti in Beih. Bot. Centralbl. XLIV. Abt. 2, 111 (1927).
Southwestern Kansu: Tao River basin, no. 12134.

Leontopodium Smithianum Hand.-Mazz. (*L. conglobatum* × *L. leontopodioides*)
Southwestern Kansu: Tao River basin, nos. 12239, 12565, 12902.

Anaphalis Hancockii Maxim.
Southwestern Kansu: Tao River basin, no. 12240.

Anaphalis lactea Maxim.
Southwestern Kansu: Tao River basin, nos. 12882, 12899.

Anaphalis margaritacea (L.) Benth. & Hook.
Central Kansu: no. 12780. Southwestern Kansu: Tao River basin, no. 13158.

Anaphalis spec.
Southwestern Kansu: Tao River basin, no. 13724.

Inula ammophila Bge.
Central Kansu: no. 13234.

Carpesium Lipskyi C. Winkl.
Southwestern Kansu: Upper Tebbu country, no. 14605.

Achillea Ptarmica L.
Southwestern Kansu: Tao River basin, no. 13667. Central Kansu: no. 12774.

Chrysanthemum fruticulosum Ledeb.
Western Kansu: no. 13258.

Chrysanthemum indicum L.
Southwestern Kansu: Tao River basin, no. 13705.

Chrysanthemum tatsienense Bur. & Franch.
Eastern Tibet: grasslands between Labrang and Yellow River, no. 14512.
Tanacetum tenuifolium Jacq.
Southwestern Kansu: Tao River basin, no. 13727; Upper Tebbu country, no. 14598. Eastern Tibet: Kokonor region, no. 13362.
Artemisia biennis Willd.
Eastern Tibet: Kokonor region, no. 13384.
Artemisia laciniata Willd.
Western Kansu: no. 13248.
Artemisia salsoloides Willd.
Eastern Tibet: Kokonor region, no. 13385.
Artemisia Sieversiana Willd.
Southwestern Kansu: Tao River basin, no. 12913.
Artemisia spec.
Southwestern Kansu: Upper Tebbu country, no. 13013.
Stereosanthus Souliei Franch.
Southwestern Kansu: Tao River basin, no. 12854.
Serratula centauroides L.
Southwestern Kansu: Tao River basin, no. 12864; Upper Tebbu country, no. 14610. Central Kansu: no. 12773.
Petasites tricholobus Franch.
Southwestern Kansu: Tao River basin, no. 12082.
Doronicum stenoglossum Maxim.
Southwestern Kansu: Tao River basin, no. 12941; Upper Tebbu country, no. 14599.
Doronicum thibetanum Cavill.
Southwestern Kansu: Tao River basin, nos. 12192, 12389; Upper Tebbu country, no. 13020.
Cacalia deltophylla (Maxim.) Mattfeld, comb. nov.
Senecio deltophylla Maxim.
Southwestern Kansu: Upper Tebbu country, no. 14586.
Cacalia Potanini (C. Winkl.) Mattfeld, comb. nov.
Senecio Potanini C. Winkl.
Southwestern Kansu: Upper Tebbu country, nos. 12998, 12998a.
Cacalia spec.
Southwestern Kansu: Tao River basin, no. 13717; Upper Tebbu country, nos. 13028, 13076, 14603. Central Kansu: no. 12722.
Senecio acerifolius C. Winkl.
Southwestern Kansu: Tao River basin, no. 12946; Upper Tebbu country, no. 14602. Central Kansu: no. 12710.
Senecio argunensis Turcz.
Southwestern Kansu: Tao River basin, nos. 13195, 13669.

Senecio campestris DC.
Southwestern Kansu: Tao River basin, nos. 12320, 12352.
Senecio nemorensis L.
Southwestern Kansu: Lower Tebbu country, no. 14856.
Senecio thianshanicus Reg. & Schmalh.
Eastern Tibet: Kokonor region, no. 13400; Radja and Yellow River gorges, no. 14214; grasslands between Labrang and Yellow River, nos. 14468, 14498.
Senecio spec.
Southwestern Kansu: Tao River basin, nos. 12836, 13139; Upper Tebbu country, nos. 12997, 14573, 14584.
Ligularia altaica DC.
Southwestern Kansu: Tao River basin, nos. 12557, 12633.
Ligularia plantaginifolia (Franch.) Mattfeld, comb. nov.
Senecio plantaginifolia Franch.
Eastern Tibet: Radja and Yellow River gorges, no. 14179; alpine region between Radja and Jupar ranges, no. 14402.
Ligularia Przewalskii Maxim.
Southwestern Kansu: Tao River basin, nos. 12846, 12907.
Ligularia sagitta (Maxim.) Mattfeld, comb. nov.
Senecio sagitta Maxim.
Southwestern Kansu: Tao River basin, nos. 13165, 14616; Upper Tebbu country, nos. 13078, 13117. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14536.
Ligularia sibirica Cass. var. **speciosa** DC.
Southwestern Kansu: Tao River basin, no. 12943; Upper Tebbu country, nos. 13126, 14592.
Ligularia tangutica (Maxim.) Mattfeld, comb. nov.
Senecio tangutica Maxim.
Southwestern Kansu: Upper Tebbu country, no. 13074
Ligularia virgaurea (Maxim.) Mattfeld, comb. nov.
Senecio virgaurea Maxim.
Southwestern Kansu: Upper Tebbu country, nos. 12999, 13038; Tao River basin, nos. 13163, 13181, 14614. Eastern Tibet: grasslands between Labrang and Yellow River, nos. 14521, 14524, 14549.
Ligularia yesoensis Franch. var. **sutchuensis** Franch.
Southwestern Kansu: Lower Tebbu country, nos. 14651, 15038.
Ligularia spec.
Southwestern Kansu: Upper Tebbu country, no. 13110.
Cremanthodium bupleurifolium W. W. Sm.
Southwestern Kansu: Tao River basin, no. 12630. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14507.
Cremanthodium Decaisnei C. B. Clarke

Eastern Tibet: grasslands between Labrang and Yellow River, no. 14464; alpine region between Radja and Jupar ranges, nos. 14159, 14393; Amnyi Machen range, no. 14428.

Cremanthodium discoideum Maxim.

Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14404.

Cremanthodium humile Maxim.

Southwestern Kansu: Upper Tebbu country, no. 13022; Tao River basin, no. 13183.

Cremanthodium Limprichtii Diels

Southwestern Kansu: Tao River basin, no. 12618.

Cremanthodium lineare Maxim.

Eastern Tibet: Kokonor region, no. 13378.

Cremanthodium plantagineum Maxim.

Southwestern Kansu: Upper Tebbu country, no. 13030. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14471; alpine region between Radja and Jupar ranges, nos. 14383, 14387; Ba Valley, no. 14357; Jupar Range, no. 14308.

Cremanthodium spec.

Southwestern Kansu: Upper Tebbu country, nos. 13012, 13049, 13084.

Echinops Turczaninowii Ledeb.

Northwestern Kansu: no. 13396.

Saussurea amara DC.

Southwestern Kansu: Tao River basin, no. 13201.

Saussurea apus Maxim.

Eastern Tibet: grasslands between Labrang and Yellow River, no. 14474.

Saussurea arenaria Maxim.

Eastern Tibet: grasslands between Labrang and Yellow River, no. 14495.

Saussurea Giraldii Diels

Central Kansu: no. 12717.

Saussurea hypsipeta Diels

Eastern Tibet: Jupar Range, no. 14367; alpine region between Radja and Jupar ranges, no. 14388; Amnyi Machen range, no. 14411.

Saussurea medusa Maxim.

Southwestern Kansu: Tao River basin, no. 12385; Upper Tebbu country, no. 13044. Eastern Tibet: alpine region between Radja and Jupar ranges, no. 14390; Jupar Range, no. 14366; Amnyi Machen range, no. 14429.

Saussurea nigrescens Maxim.
Southwestern Kansu: Upper Tebbu country, no. 13067.
Saussurea phaeantha Maxim.
Eastern Tibet: grasslands between Labrang and Yellow River, no. 14516.
Saussurea polystichoides Hook. f.
Southwestern Kansu: Tao River basin, no. 13737.
Saussurea poophylla Diels
Southwestern Kansu: Upper Tebbu country, no. 13001.
Saussurea pygmaea Spreng. var.
Eastern Tibet: grasslands between Labrang and Yellow River, no. 14458.
Saussurea stella Maxim.
Eastern Tibet: Kokonor region, nos. 13349, 13379. Southwestern Kansu: Tao River basin, no. 13666.
Saussurea Thoroldi Hemsl.
Eastern Tibet: Kokonor region, nos. 13376, 13377.
Saussurea spec. aff. *S. poophylla* Diels
Central Kansu: no. 12721.
Saussurea spec.
Southwestern Kansu: Tao River basin, nos. 13156, 13180, 13199, 13200, 13739, 14617, 14618; Upper Tebbu country, nos. 13034, 13036, 13121, 14604, 14626; Lower Tebbu country, no. 14817. Central Kansu: nos. 12740, 12771. Northwestern Kansu: 13386. Eastern Tibet: Kokonor region, nos. 13700, 13702; grasslands between Labrang and Yellow River, no. 14485; Jupar Range, nos. 14337, 14368; Amnyi Machen range, nos. 14413, 14425, 14433.
Carduus euosmos Forrest apud W. W. Sm.
Eastern Tibet: Kokonor region, no. 13371. Southwestern Kansu: Upper Tebbu country, no. 14582.
Cirsium arvense Scop.
Eastern Tibet: Amnyi Machen range, no. 14443.
Cirsium setosum Bieb.
Central Kansu: no. 12764. Southwestern Kansu: Tao River basin, no. 13718.
Cirsium Souliei (Franch.) Mattfeld, comb. nov.
Cnicus Souliei Franch.
Eastern Tibet: grasslands between Labrang and Yellow River, no. 14496.
Cirsium spec.
Southwestern Kansu: Upper Tebbu country, no. 13122.
Scorzonera austriaca Willd.

Southwestern Kansu: Tao River basin, no. 12169. Eastern Tibet: Radja and Yellow River gorges, no. 14104.

Taraxacum mongolicum Hand.-Mazz.

Eastern Tibet: Radja and Yellow River gorges, no. 13979.

Lactuca spec. aff. *L. Souliei* Franch.

Southwestern Kansu: Tao River basin, no. 13729.

Crepis Hookeriana C. B. Clarke

Southwestern Kansu: Tao River basin, no. 12645. Eastern Tibet: grasslands between Labrang and Yellow River, no. 14517; alpine region between Radja and Jupar ranges, no. 14386; Amnyi Machen range, no. 14442.

Crepis paleacea Diels aff.

Southwestern Kansu: Upper Tebbu country, no. 13062.

Crepis rosularis Diels aff.

Southwestern Kansu: Upper Tebbu country, no. 13025.

Crepis spec. aff. *C. trichocarpa* Franch.

Eastern Tibet: grasslands between Labrang and Yellow River, no. 14529.

Prenanthes spec.

Southwestern Kansu: Upper Tebbu country, no. 14591.

LIST OF SEEDS OF LIGNEOUS AND HERBACEOUS PLANTS COLLECTED IN 1925 AND 1926 BY J. F. ROCK AND DISTRIBUTED BY THE ARNOLD ARBORETUM

12131. Pyrus pashia Ham.
12141. Viburnum fragrans Bge.
12193. Lonicera hispida Pall.
12270. Lonicera syringantha Maxim.
12297. Lonicera syringantha Maxim.
12432. Prunus setulosa Batal.
12475. Lonicera hispida Pall.
12527. Prunus stipulacea Maxim.
12532. Lonicera tangutica Maxim.
12797. Berberis parvifolia Sprague
12893. Lonicera coerulea L. var. edulis Reg.
13197. Ribes stenocarpum Maxim.
13202. Berberis Mouillacana Schneid.
13225. Prinsepia uniflora Batal.
13240. Nitraria Schoberi L.
13243. Ailanthus altissima (Mill.) Swingle
13244. Ampelopsis aconitifolia Bge. var. palmiloba (Carr.) Rehd.
13256. Berberis Vernae Schneid.
13257. Lycium chinense Mill.
13261. Berberis Boschanii Schneid.
13262. Berberis dasystachya Maxim.
13263. Berberis diaphana Maxim.
13264. Rosa Sweginzowii Koehne
13265. Rosa bella Rehd. & Wils.
13266. Rosa Sweginzowii Koehne
13267. Sorbus thianschanica Rupr.
13268. Sorbus Koehneana Schneid.
13269. Cotoneaster tenuipes Rehd. & Wils.
13270. Hippophoë rhamnoides L.
13271. Rosa omeiensis Rolfe
13272. Berberis diaphana Maxim.
13273. Berberis diaphana Maxim.
13274. Berberis Boschanii Schneid.

13275. Hippophoë rhamnoides L.
13276. Lonicera syringantha Maxim.
13277. Clematis tangutica Korsh. var. obtusiuscula Rehd. & Wils.
13278. Rhododendron Przewalskii Maxim.
13279. Rhododendron anthopogonoides Maxim.
13280. Caragana brevifolia Komar.
13281. Picea asperata Mast.
13282. Picea asperata Mast.
13283. Betula japonica Sieb. var. Rockii Rehd.
13285. Rubus idaeus L. var. strigosus Maxim.
13286. Sorbus thianschanica Rupr.
13288. Lonicera nervosa Maxim.
13289. Berberis Vernae Schneid.
13290. Berberis kansuensis Schneid.
13291. Berberis dasystachya Maxim.
13292. Berberis kansuensis Schneid.
13293. Rosa bella Rehd. & Wils.
13294. Cotoneaster acutifolia Turcz.
13295. Berberis diaphana Maxim.
13296. Cotoneaster multiflora Bge.
13297. Rhamnus leptophylla Schneid. var. scabrella Rehd.
13298. Lonicera syringantha Maxim.
13299. Rosa Willmottiae Hemsl.
13300. Berberis Boschanii Schneid.
13301. Incarvillea compacta Maxim.
13302. Rhododendron Przewalskii Maxim.
13303. Rhododendron thymifolium Maxim.
13304. Picea asperata Mast.
13305. Juniperus zaidamensis Komar.
13307. Picea asperata Mast.
13308. Clematis brevicaudata DC.
13309. Picea asperata Mast.
13310. Picea asperata Mast.
13311. Evonymus Przewalskii Maxim.
13313. Picea asperata Mast.
13314. Berberis Caroli Schneid.
13315. Berberis Vernae Schneid.
13316. Berberis Boschanii Schneid.
13317. Sophora alopecuroides L.
13318. Sorbus thianschanica Rupr.
13319. Elaeagnus angustifolia L.
13320. Nitraria Schoberi L.
13320a. Zygophyllum xanthoxylum Maxim.
13321. Potentilla Salesoviana Steph.
13322. Clematis fruticosa Turcz. var. canescens Turcz.
13341. Picea asperata Mast.
13422. Abies Faxoniana Rehd. & Wils.
13423. Abies Faxoniana Rehd. & Wils.
13424. Picea purpurea Mast.
13425. Abies Faxoniana Rehd. & Wils.
13426. Picea Wilsonii Mast.
13427. Picea purpurea Mast.
13428. Picea asperata Mast.
13429. Abies Faxoniana Rehd. & Wils.
13430. Picea purpurea Mast.
13431. Picea asperata Mast.
13432. Juniperus formosana Hay.
13433. Picea asperata Mast.
13434. Picea purpurea Mast.
13435. Abies sutchuenensis (Franch.) Rehd. & Wils.
13436. Abies Faxoniana Rehd. & Wils.
13437. Abies Faxoniana Rehd. & Wils.
13438. Picea asperata Mast.
13439. Picea purpurea Mast.
13440. Abies Faxoniana Rehd. & Wils.
13441. Picea purpurea Mast.
13442. Picea purpurea Mast.
13443. Picea Wilsonii Mast.
13444. Abies Faxoniana Rehd. & Wils.
13445. Abies Faxoniana Rehd. & Wils.

13446. Picea asperata Mast.
13447. Abies Faxoniana Rehd. & Wils.
13448. Abies Faxoniana Rehd. & Wils.
13449. Pinus tabulaeformis Carr.
13450. Abies sutchuenensis (Franch.) Rehd. & Wils.
13451. Abies sutchuenensis (Franch.) Rehd. & Wils.
13452. Picea Wilsonii Mast.
13453. Picea Wilsonii Mast.
13454. Abies sutchuenensis (Franch.) Rehd. & Wils.
13455. Picea purpurea Mast.
13456. Abies sutchuenensis (Franch.) Rehd. & Wils.
13457. Picea Wilsonii Mast.
13458. Picea purpurea Mast.
13459. Picea asperata Mast.
13460. Picea purpurea Mast.
13461. Pinus tabulaeformis Carr.
13462. Picea purpurea Mast.
13463. Pinus Armandi Franch.
13464. Picea asperata Mast.
13465. Larix Potaninii Batal.
13466. Abies Faxoniana Rehd. & Wils.
13467. Abies sutchuenensis (Franch.) Rehd. & Wils.
13468. Juniperus saltuaria Rehd. & Wils.
13469. Juniperus distans Florin
13470. Juniperus squamata Buch.-Ham. var. Fargesii Rehd. & Wils.
13471. Juniperus saltuaria Rehd. & Wils.
13472. Picea purpurea Mast.
13473. Viburnum betulifolium Batal.
13474. Berberis diaphana Maxim.
13475. Cotoneaster adpressa Bois
13476. Viburnum betulifolium Batal.
13477. Sorbus Koehneana Schneid.
13478. Cotoneaster horizontalis Decne.
13479. Sorbus hupehensis Schneid. var. aperta (Koehne) Schneid.
13480. Cotoneaster multiflora Bge. var. calocarpa Rehd. & Wils.
13481. Aralia chinensis L. var. nuda Nakai
13482. Quercus liaotungensis Koidz.
13483. Cornus macrophylla Wall.
13484. Tilia chinensis Maxim.
13485. Virburnum Sargenti Koehne var. calvescens Rehd.
13486. Tilia chinensis Maxim.
13487. Quercus liaotungensis Koidz.
13488. Acer Maximowiczii Pax
13489. Acer tetramerum Pax var. betulifolium Rehd.
13490. Clematoclethra lasiocladą Maxim.
13491. Acanthopanax Giraldii Harms
13492. Viburnum Sargenti Koehne var. calvescens Rehd.
13493. Cotoneaster multiflora Bge. var. calocarpa Rehd. & Wils.
13494. Sorbus Prattii Koehne
13495. Caragana frutex K. Koch
13495a. Rosa Sweginzowii Koehne
13496. Pyrus pashia Ham.
13497. Malus kansuensis Schneid.
13498. Malus baccata Borkh.
13499. Pyrus ussuriensis Maxim. var. ovoidea Rehd.
13500. Crataegus kansuensis Wils.
13501. Crataegus kansuensis Wils.
13502. Malus baccata Borkh.
13503. Celtis Bungeana Bl.
13504. Prinsepia uniflora Batal.
13505. Ribes Giraldii Jancz.
13506. Syringa pekinensis Rupr.
13507. Syringa pekinensis Rupr.
13508. Evonymus phellomana Loes.
13509. Lonicera heteroloba Batal.

13510. Daphne Giraldii Nitsche
13511. Corylus Sieboldiana Bl. var. mandshurica Schneid.
13512. Lonicera chrysantha Turcz. var. longipes Maxim.
13513. Ostryopsis Davidiana Decne.
13514. Berberis dasystachya Maxim.
13515. Viburnum mongolicum (Pall.) Rehd.
13516. Lonicera chrysantha Turcz. var. longipes Maxim.
13517. Cotoneaster multiflora Bge. Maxim.
13518. Viburnum glomeratum
13519. Lonicera Ferdinandi Franch.
13520. Lonicera chrysantha Turcz. var. longipes Maxim.
13521. Evonymus phellomana Loes.
13522. Berberis Silva-Taroucana Schneid.
13523. Cotoneaster racemiflora K. Koch var. soongarica Schneid.
13524. Sorbus Koehneana Schneid.
13525. Berberis diaphana Maxim.
13526. Berberis kansuensis Schneid.
13527. Prunus salicina Lindl.
13528. Berberis kansuensis Schneid.
13529. Berberis diaphana Maxim.
13530. Berberis parvifolia Sprague
13531. Daphne tangutica Maxim.
13532. Sorbus tapashana Schneid.
13533. Caragana jubata Poiret
13534. Acanthopanax Giraldii Harms
13535. Cotoneaster adpressa Bois
13536. Ribes Meyeri Maxim.
13537. Rosa omeiensis Rolfe
13538. Caragana Maximovicziana Komar.
13539. Malus transitoria (Batal.) Schneid.
13540. Sorbus Prattii Koehne
13541. Caragana jubata Poiret
13542. Acer tetramerum Pax var. betulifolium Rehd.
13543. Malus toringoides (Rehd.) Hughes
13544. Malus toringoides (Rehd.) Hughes
13545. Berberis brachypoda Maxim.
13546. Rubus pileatus Focke
13547. Rosa omeiensis Rolfe
13548. Cotoneaster acutifolia Turcz.
13549. Pyrus pashia Ham.
13550. Lonicera syringantha Maxim.
13551. Lonicera coerulea L. var. edulis Reg.
13553. Rubus idaeus L. var. strigosus Maxim.
13554. Evonymus nanoides Loes. & Rehd.
13555. Prunus tangutica (Batal.) Koehne
13556. Lonicera heteroloba Batal.
13557. Berberis Vernae Schneid.
13558. Pyrus pashia Ham.
13559. Malus toringoides (Rehd.) Hughes
13560. Lycium chinense Mill.
13561. Berberis dasystachya Maxim.
13562. Caragana brevifolia Komar.
13563. Sorbus Prattii Koehne
13564. Berberis parvifolia Sprague
13565. Malus toringoides (Rehd.) Hughes
13566. Sorbus tapashana Schneid.
13567. Caragana jubata Poiret
13568. Malus toringoides (Rehd.) Hughes
13569. Evonymus Giraldii Loes. var. ciliata Loes.
13570. Rosa Sweginzowii Koehne[1]
13571. Hippophoë rhamnoides L.
13572. Daphne tangutica Maxim.
13573. Rosa Sweginzowii Koehne

[1]Under this number two sets of seeds were distributed. One set corresponded to the species cited above while the second set proved to be seed of *Prunus Padus* L. var. *commutata* Dipp.

13574. Berberis diaphana Maxim.
13575. Rosa omeiensis Rolfe
13576. Lonicera deflexicalyx Batal.
13577. Prunus Padus L. var. commutata Dipp.
13578. Lonicera chrysantha Turcz. var. longipes Maxim.
13579. Ribes Meyeri Maxim.
13580. Lonicera tangutica Maxim. var. glabra Batal.
13581. Rubus amabilis Focke
13582. Lonicera nervosa Maxim.
13583. Lonicera thibetica Bur. & Franch.
13584. Lonicera hispida Pall.
13585. Lonicera heteroloba Batal.
13586. Ribes Meyeri Maxim.
13587. Evonymus Przewalskii Maxim.
13588. Lonicera deflexicalyx Batal.
13589. Sorbus Koehneana Schneid.
13590. Rosa omeiensis Rolfe
13591. Rosa Willmottiae Hemsl.
13592. Lonicera szechuanica Batal.
13593. Paeonia anomala L.
13594. Spiraea gemmata Zab.
13595. Sibiraea laevigata Maxim. var. angustata Rehd.
13596. Rhododendron capitatum Maxim.
13597. Rhododendron anthropogonoides Maxim.
13598. Rhododendron thymifolium Maxim.
13599. Rhododendron rufum Batal.
13600. Rhododendron capitatum Maxim.
13601. Rhododendron rufum Batal.
13602. Hydrangea Bretschneideri Dipp.
13603. Philadelphus pekinensis Rupr. var. kansuensis Rehd.
13604. Hydrangea Bretschneideri Dipp.
13605. Rhododendron capitatum Maxim.
13606. Lilium tenuifolium Fisch. & Mey.
13607. Acer caudatum Wall. var. multiserratum Rehd.
13608. Rhamnus leptophylla Schneid. var. scabrella Rehd.
13609. Arctous ruber Nakai
13610. Rhododendron anthopogonoides Maxim.
13611. Rhododendron capitatum Maxim.
13612. Rhododendron Przewalskii Maxim.
13613. Rhododendron rufum Batal.
13614. Clematis brevicaudata DC.
13615. Clematis tangutica Korsh. var. obtusiuscula Rehd. & Wils.
13616. Betula albo-sinensis Burk.
13617. Buddleia alternifolia Maxim.
13618. Lilium Duchartrei Franch.
13619. Primula tangutica Duthie.
13620. Meconopsis quintuplinervia Reg.
13621. Betula albo-sinensis Burk.
13622. Rhododendron capitatum Maxim.
13624. Clematis Fargesii Franch.
13625. Anemone narcissiflora L.
13626. Anemone Rockii Ulbrich
13627. Meconopsis racemosa Maxim.
13628. Rhododendron rufum Batal.
13629. Rhododendron Przewalskii Maxim.
13630. Rhododendron rufum Batal.
13631. Primula? chionantha Balf. f. & Forrest.
13632. Meconopsis psilonomma Farrer
13633. Primula? optata Balf. f. & Farrer
13634. Rhododendron capitatum Maxim.
13635. Rhododendron capitatum Maxim.
13636. Rhododendron anthopogonoides Maxim.
13637. Iris ensata Thunb.
13638. Caragana densa Komar.
13639. Primula flava Maxim.
13640. Rhododendron rufum Batal.
13641. Betula albo-sinensis Burk.

13642. Meconopsis punicea Maxim.
13643. Rhododendron rufum Batal.
13644. Betula japonica Sieb. var. szechuanica Schneid.
13645. Rhododendron rufum Batal.
13646. Betula albo-sinensis Burk.
13647. Rhododendron rufum Batal.
13648. Betula albo-sinensis Burk. var. septentrionalis Schneid.
13649. Rhododendron rufum Batal.
13650. Rhododendron rufum Batal.
13651. Meconopsis integrifolia Franch.
13652. Primula polyneura Franch.
13653. Incarvillea compacta Maxim.
13654. Primula limbata Balf. f. & Forrest
13655. Primula tangutica Duthie
13656. Primula chionantha Balf. f. & Forrest
13657. Betula japonica Sieb. var. szechuanica Schneid.
13658. Primula ? Woodwardii Balf. f.
13659. Philadelphus pekinensis Rupr. var. kansuensis Rehd.
13670. Sorbaria arborea Schneid. var. glabrata Rehd.
13671. Hydrangea Bretschneideri Dipp.
13672. Potentilla fruticosa L. var. parvifolia Wolf
13673. Potentilla fruticosa L. var. dahurica Ser.
13674. Rhododendron capitatum Maxim.
13675. Rhododendron rufum Batal.
13676. Rhododendron Przewalskii Maxim.
13677. Rhododendron Przewalskii Maxim.
13678. Rhododendron rufum Batal.
13679. Rhododendron Przewalskii Maxim.
13680. Rhododendron rufum Batal.
13681. Rhododendron Przewalskii Maxim.
13682. Rhododendron rufum Batal.
13683. Rhododendron rufum Batal.
13684. Rhododendron rufum Batal.
13685. Rhododendron Przewalskii Maxim.
13686. Rhododendron Przewalskii Maxim.
13687. Philadelphus pekinensis Rupr. var. kansuensis Rehd.
13688. Rhododendron capitatum Maxim.
13689. Spiraea alpina Pall.
13690. Spiraea longigemmis Maxim. (pubescent form)
13691. Rhododendron rufum Batal.
13692. Rhododendron rufum Batal.
13693. Rhododendron rufum Batal.
13694. Rhododendron Przewalskii Maxim.
13695. Rhododendron Przewalskii Maxim.
13696. Rhododendron rufum Batal.
13697. Rhododendron rufum Batal.
13698. Syringa microphylla Diels
13699. Picea asperata Mast.
13744. Berberis parvifolia Sprague
13918. Juniperus glaucescens Florin
13946. Juniperus tibetica Komar.
13963. Picea asperata Mast.
14564. Prinsepia uniflora Batal.
14570. Prunus salicina Lindl.
14577. Cotoneaster multiflora Bge.
14581. Rhamnus leptophylla Schneid.
14662. Cornus macrophylla Wall.
14667. Meliosma cuneifolia Franch.
14673. Aralia chinensis L. var. nuda Nakai
14718. Betula japonica Sieb. var. szechuanica Schneid.
14734. Lonicera deflexicalyx Batal.
14736. Lonicera chrysantha Turcz. var. longipes Maxim.
14794. Lonicera Ferdinandi Franch.
14801. Cotoneaster multiflora Bge.
14802. Crataegus kansuensis Wils. f. aurantiaca Wils.

14804. Berberis kansuensis Schneid.
14805. Betula japonica Sieb. var. szechuanica Schneid.
14806. Cotoneaster nitens Rehd. & Wils.
14807. Pyrus pashia Ham.
14810. Lonicera szechuanica Batal.
14816. Pyrus pashia Ham.
14823. Betula albo-sinensis Burk. var. septentrionalis Schneid.
14825. Aralia chinensis L. var. nuda Nakai
14829. Cotoneaster Zabeli Schneid.
14834. Cotoneaster obscura Rehd. & Wils. var. cornifolia Rehd. & Wils.
14857. Prunus pubigera Koehne var. Prattii Koehne
14860. Aconitum volubile Pall. var. flexuosus Rchb.
14864. Juniperus distans Florin
14866. Acanthopanax Giraldii Harms
14875. Juniperus distans Florin
14876. Lonicera nervosa Maxim.
14877. Ribes Vilmorini Jancz.
14882. Rosa Sweginzowii Koehne
14883. Prunus pubigera Koehne var. Prattii Koehne
14888. Berberis Vernae Schneid.
14889. Prunus tangutica Koehne
14890. Lonicera heteroloba Batal.
14891. Lonicera deflexicalyx Batal.
14892. Rosa Willmottiae Hemsl.
14893. Prunus Padus L. var. commutata Dipp.
14894. Daphne tangutica Maxim.
14895. Rubus amabilis Focke
14896. Rosa Sweginzowii Koehne
14897. Cotoneaster multiflora Bge.
14898. Berberis Mouillacana Schneid.
14899. Berberis kansuensis Schneid.
14900. Rosa omeiensis Rolfe
14901. Podophyllum emodi Wall.
14902. Sorbus Prattii Koehne
14903. Rosa Sweginzowii Koehne
14905. Cotoneaster multiflora Bge.
14906. Lonicera Ferdinandi Franch.
14907. Rosa Sweginzowii Koehne
14908. Crataegus kansuensis Wils.
14909. Prunus salicina Lindl.
14910. Malus transitoria Schneid.
14911. Berberis parvifolia Sprague
14912. Rosa Willmottiae Hemsl.
14913. Berberis Silva-Taroucana Schneid.
14914. Cotoneaster multiflora Bge.
14915. Berberis Vernae Schneid.
14916. Betula japonica Sieb. var. szechuanica Schneid.
14917. Crataegus kansuensis Wils.
14918. Malus kansuensis Schneid.
14919. Sorbus Prattii Koehne
14920. Primula ? chionantha Balf. f. & Forrest forma
14922. Malus transitoria Schneid.
14923. Malus toringoides (Rehd.) Hughes
14924. Pyrus pashia Ham.
14925. Malus kansuensis × toringoides Rehd.
14926. Philadelphus pekinensis Rupr. var. kansuensis Rehd.
14927. Rosa Sweginzowii Koehne
14928. Rhododendron rufum Batal.
14929. Abies Faxoniana Rehd. & Wils.
14930. Abies sutchuenensis (Franch.) Rehd. & Wils.
14931. Abies sutchuenensis (Franch.) Rehd. & Wils.
14933. Caragana jubata Poiret
14938. Malus kansuensis Schneid.
14939. Crataegus kansuensis Wils.
14940. Quercus Baronii Skan
14941. Quercus liaotungensis Koidz.
14942. Quercus Baronii Skan
14943. Berberis Vernae Schneid.
14944. Rosa Willmottiae Hemsl.
14945. Clematoclethra lasioclada Maxim.
14946. Acer Maximowiczii Pax

14947. Ribes moupinense Franch. var. tripartitum Jancz.
14948. Philadelphus pekinensis Rupr. var. kansuensis Rehd.
14949. Acer tetramerum Pax var. betulifolium Rehd.
14950. Quercus Baronii Skan.
14951. Juniperus chinensis L. var. pendula Franch.
14952. Juniperus chinensis L.
14953. Ribes Giraldii Jancz.
14954. Lonicera Ferdinandi Franch.
14955. Evonymus nanoides Loes. & Rehd.
14956. Crataegus kansuensis Wils.
14957. Prinsepia uniflora Batal.
14958. Sageretia theezans Brongn.
14959. Quercus liaotungensis Koidz.
14960. Lonicera Ferdinandi Franch.
14964. Picea Wilsonii Mast.
14965. Caragana tangutica Maxim.
14966. Betula albo-sinensis Burk. var. septentrionalis Schneid.
14967. Quercus liaotungensis Koidz.
14968. Cornus macrophylla Wall.
14969. Betula albo-sinensis Burk. var. septentrionalis Schneid.
14970. Clematoclethra lasioclada Maxim.
14971. Viburnum betulifolium Batal. f. aurantiacum Rehd.
14972. Cotoneaster adpressa Bois
14973. Caragana densa Komar.
14974. Quercus liaotungensis Koidz.
14975. Picea Wilsonii Mast.
14976. Hydrangea Bretschneideri Dipp.
14977. Lonicera nervosa Maxim.
14978. Evonymus alata Reg. var. aperta Loes.
14979. Rubus pileatus Focke
14980. Cotoneaster lucida Schlecht.
14981. Cotoneaster nitens Rehd. & Wils.
14982. Spiraea Wilsonii Duthie
14983. Acanthopanax Giraldii Harms
14984. Acer tetramerum Pax var. betulifolium Rehd.
14985. Clematoclethra integrifolia Maxim.
14986. Viburnum glomeratum Maxim.
14987. Sorbus Koehneana Schneid.
14988. Rosa Sweginzowii Koehne
14989. Abies Faxoniana Rehd. & Wils.
14990. Sorbus hupehensis Schneid. var. aperta (Koehne) Schneid.
14991. Hippophoë rhamnoides L.
14992. Cotoneaster multiflora Bge. var. calocarpa Rehd. & Wils.
14993. Lonicera chrysantha Turcz. var. longipes Maxim.
14994. Koelreuteria paniculata Laxm.
14995. Quercus Baronii Skan
14996. Exochorda Giraldii Hesse
14997. Acer tetramerum Pax var. betulifolium Rehd.
14998. Acer Maximowiczii Pax
14999. Tilia chinensis Maxim.
15000. Acer pictum Thbg. var. parviflorum Schneid.
15001. Tilia chinensis Maxim.
15002. Quercus liaotungensis Koidz.
15003. Viburnum glomeratum Maxim. var. Rockii Rehd.
15004. Rhododendron micranthum Turcz.
15005. Hydrangea Bretschneideri Dipp. var. glabrescens Rehd.
15006. Wikstroemia chamaedaphne Meisn.
15007. Clematis fruticosa Turcz.
15008. Clematis aethusifolia Turcz.

15009. Abies Faxoniana Rehd. & Wils.
15010. Acer caudatum Wall. var. multiserratum Rehd.
15011. Viburnum betulifolium Batal.
15012. Buddleia albiflora Hemsl.
15013. Viburnum betulifolium Batal.
15014. Rhododendron rufum Batal
15015. Clematoclethra lasioclada Maxim.
15016. Hydrangea longipes Franch.
15017. Betula japonica Sieb. var. szechuanica Schneid.
15018. Betula japonica Sieb. var. szechuanica Schneid.
15019. Pinus Armandi Franch.
15020. Abies sutchuenensis (Franch.) Rehd. & Wils.
15021. Picea Wilsonii Mast.
15022. Lonicera chrysantha Turcz. var. longipes Maxim.
15023. Acanthopanax leucorrhizus Harms
15024. Sorbus hupehensis Schneid. var. aperta (Koehne) Schneid.
15025. Sorbus Koehneana Schneid.
15026. Actinidia tetramera Maxim.
15028. Viburnum betulifolium Batal.
15029. Berberis brachypoda Maxim.
15030. Quercus liaotungensis Koidz.
15031. Acer Maximowiczii Pax
15032. Acer tetramerum Pax. var. betulifolium Rehd.
15033. Acer Davidi Franch.
15034. Tilia chinensis Maxim.
15035. Jasminum humile L.
15036. Syringa oblata Lindl. var. Giraldii Rehd.
15037. Quercus Baronii Skan
15038. Ligularia yesoensis Franch. var. sutchuensis Franch.
15039. Hydrangea Bretschneideri Dipp. var. glabrescens Rehd.
15040. Acer caudatum Wall. var. multiserratum Rehd.
15041. Acer Maximowiczii Pax
15042. Tilia chinensis Maxim.
15043. Pyrus pashia Ham.
15044. Abies Faxoniana Rehd. & Wils.
15045. Picea asperata Mast.
15046. Philadelphus pekinensis Rupr. var. kansuensis Rehd.
15047. Acer Maximowiczii Pax
15048. Picea Wilsonii Mast.
15049. Spiraea uratensis Franch.
15050. Rhamnus leptophylla Schneid.
15051. Acer tetramerum Pax var. betulifolium (Maxim.) Rehd.
15052. Tilia chinensis Maxim.
15053. Acer Grosseri Pax
15054. Pyrus pashia Ham.
15055. Aster Limprichtii Diels
15056. Indigofera Bungeana Walp.
15058. Syringa oblata Lindl. var. Giraldii (Sprenger) Rehd.
15059. Exochorda Giraldii Hesse
15060. Ailanthus altissima (Mill.) Swingle
15061. Caragana densa Komar.
15062. Caragana tangutica Maxim.
15063. Syringa oblata Lindl. var. Giraldii (Sprenger) Rehd.
15064. Picea Wilsonii Mast.
15065. Picea asperata Mast.
15066. Juniperus squamata Buch.-Ham. var. Fargesii Rehd. & Wils.
15067. Smilax rubriflora Rehd.
15068. Picea Wilsonii Mast.
15069. Tilia chinensis Maxim.
15070. Syringa pekinensis Rupr.
15071. Buddleia albiflora Hemsl.
15072. Evonymus phellomana Loes.
15073. Quercus liaotungensis Koidz.
15074. Malus toringoides (Rehd.) Hughes

15075. Quercus liaotungensis Koidz.
15076. Philadelphus pekinensis Rupr. var. kansuensis Rehd.
15077. Pertya sinensis Oliver
15078. Lonicera Ferdinandi Franch.
15079. Abies sutchuenensis (Franch.) Rehd. & Wils.
15080. Picea asperata Mast.
15081. Abies Faxoniana Rehd. & Wils.
15082. Abies Faxoniana Rehd. & Wils.
15083. Betula albo-sinensis Burk.
15084. Abies Faxoniana Rehd. & Wils.
15085. Aralia chinensis L. var. nuda Nakai
15086. Picea Wilsonii Mast.
15087. Abies recurvata Mast.
15088. Abies recurvata Mast.
15089. Picea Wilsonii Mast.
15090. Pyrus pashia Ham.
15091. Picea Wilsonii Mast.
15092. Picea asperata Mast.
15093. Juniperus squamata Buch.-Ham. var. Fargesii Rehd. & Wils.
15094. Lycium chinense Mill.
15095. Bocconia spec.

HERBARIUM, ARNOLD ARBORETUM
HARVARD UNIVERSITY

CONTRIBUTION TO THE FLORA OF THE NEW HEBRIDES PLANTS COLLECTED BY S. F. KAJEWSKI IN 1928 AND 1929

SUPPLEMENT[1]

A. Guillaumin

MENISPERMACEAE

Pycnarrhena sp.

Banks Group: Vanua Lava Island, rain-forest, sea level, no. **410**, July 5, 1928 (vine growing on rain-forest trees; fruit orange when ripe).

The eastern limit of this genus had been until now New Guinea and Queensland.

CAPPARIDACEAE

Capparis sp. aff. *C. artensis* Montr. (Art Island) vel *C. subacuta* Miq. (Borneo).

Efate: Undine Bay, common at sea level, no. **228**, April 28, 1928 (vine climbing on top of trees; fruit purple when ripe).

FLACOURTIACEAE

Flacourtia sp. (teste E. D. Merrill).

Aneityum: Anelgauhat Bay, common in rain-forest, alt. 120 m., no. **812**, Feb. 23, 1929 (large tree up to 18 m. high; fruit yellow when ripe).

Xylosma sp. aff. *X. lifuana* Guillaumin (Loyalty Islands).

Aneityum: Anelgauhat Bay, common in rain-forest, alt. 60 m., no. **745**, Feb. 12, 1929 (small tree up to 6 m. high; flowers greenish yellow).

PITTOSPORACEAE

Pittosporum naruaiao Guillaumin, sp. nov.

Arbor, trunco 1 m. diam., ramis gracilibus fulvis, foliis ad ramulorum apicem confertis lanceolatis (usque ad 14 cm. × 3 cm.) apice acutis acuminatisve basi cuneatis membranaceis, nervis parum conspicuis, petiolo gracili 1-2 cm. longo. Inflorescentiae umbellatae, 2-2.5 cm. longae, pedunculo rufo-puberulo, bracteis parvis triangularibus extra rufo-puberulis, pedicellis 5 mm. longis rufo-puberulis, floribus albis (tantum in alabastris 3 mm. longis), calyce glabro campanulato 1 mm. longo, lobis ovatis tubo 2-plo brevioribus, petalis ovatis 1.7 mm. longis, staminibus petalis fere aequilongis, filamentis antheris 2-plo brevioribus

[1]See Vol. XII. 221-264, XIII. 1-29, 81-126 for the preceding parts.

complanatis ovatis, antheris linearibus apice apiculatis, ovario turbinato staminibus fere aquilongo. Fructus rubri (4 cm. x 2.5 cm.)

A n e i t y u m: west side, Nowhow, common in rain-forest, alt. 600 m., no. **1004** (coll. *J. P. Wilson*), Sept. 1929 (large tree, up to 1 m. in diameter; flowers white; fruit red). Vernacular name "Naruaiao."

GUTTIFERAE

Garcinia sp.

E f a t e: Undine Bay, common in rain-forest, alt. 100 m., no. **213**, April 26, 1928 (tree 15 m. high; fruit orange).

TERNSTROEMIACEAE

Eurya japonica Thunberg, Nov. Gen. 67 (1781).

A n e i t y u m: Anelgauhat Bay, common in rain-forest, alt. 60 m., no. **747**, Feb. 12, 1929 (small tree up to 6 m. high; petals white; fruit black when ripe). T a n n a: Mt. Tokosh Meru, common in rain-forest, alt. 300 m., no. **153**, March 15, 1928 (tree up to 15 m. high).—Already found on Aneityum, (*MacGillivray* 58)[1] also in the Fiji, Caroline and Palau Islands and in Malaysia.

STERCULIACEAE

Kleinhovia hospita Linnaeus, Sp. Pl. ed. 2, 1365 (1763).

E f a t e: Undine Bay, common in rain-forest at sea-level, no. **199**, April 24, 1928 (shade tree with dense leaves 15-20 m. high; flowers pink).—Already found on Aneityum and Efate; also Fiji, Society, Solomon and Bismarck Islands, New Guinea and Malaysia.

ELAEOCARPACEAE

Elaeocarpus sp.

A n e i t y u m: Anelgauhat Bay, common in rain-forest, alt. 300 m., no. **938**. March 17, 1929 (medium sized tree up to 15 m. high).

BURSERACEAE

Canarium aneityense Guillaumin, sp. nov.

Arbor magna, foliis usque ad 28 cm. longis, foliolis 3-5 ovatis apice longe caudato-acuminatis basi obtusis rotundatis truncatisve rigidis, nervis 7-jugis, petiolulis terminali 4 cm., lateralibus 2 cm. longis apice tumidis, petiolo 3.5-6 cm. longo, stipulis ?. Inflorescentiae ad apicem axillares, paniculatae, 13 cm. longae, ramis gracilibus inferioribus 8 cm. longis, floribus albo-viridibus, ♂ minutis, pedicello 2-3 mm. longo medio articulato basi minute bracteolato, sepalis ovato-triangularibus 2 mm. longis, petalis ovatis aequilongis, staminibus filamentis brevissi-

[1]Not mentioned in my "Liste des plantes connues [des Nouvelles Hebrides] in Bull. Soc. Bot. France, LXXIV (1927).

mis, antheris petalis 2-plo brevioribus, disco annulari, pistillodio minimo, stylo terete brevi.

A n e i t y u m: Anelgauhat Bay, common in rain-forest, alt. 150 m., no. **943**; March 19, 1929 (large tree; flowers green-white).—Valuable cabinet wood; vernacular name "Inyat."

MELIACEAE

Melia Azedarach Linnaeus, Sp. Pl. 384 (1753).

A n e i t y u m: Anelgauhat Bay, common in rain-forest at sea level, no. **897,** March 11, 1929 (large tree up to 18 m. high; fruit red when ripe).—Vernacular name "White Cedar."

DICHAPETALACEAE

Dichapetalum sp. aff. *D. missionum* Krause (Bismarck Islands) vel *D. validum* Krause (New Guinea).

A n e i t y u m: Anelgauhat Bay, common in rain-forest, alt. 300 m., no. **763,** Feb. 14, 1929 (vine climbing up rain-forest trees; fruit brown when ripe).

OLACACEAE

Ximenia americana Linnaeus, Sp. Pl. 1193 (1753).

A n e i t y u m: Anelgauhat Bay, common along sea shore at sea level; no. **797,** Feb. 21, 1929 (rambling tree growing to the height of 4.5 m.; fruit yellow when ripe, with almond taste).—Also in New Caledonia, Loyalty, Fiji, Society and Mariana Islands, New Guinea, Australia (Queensland) and Malaysia.

AQUIFOLIACEAE

Ilex sp. aff. *I. peduncularis* F. Muell. (Queensland).

A n e i t y u m: Anelgauhat Bay, common in rain-forest, alt. 60 m., no. **746,** Feb. 12, 1929 (large tree up to 18 m. high; fruit red when ripe).

HIPPOCRATEACEAE

Salacia aneityensis Guillaumin, sp. nov.

Scandens, glaberrimus etiam novellis, foliis atro-viridibus subdiscoideis (usque ad 11 cm. x 8 cm.) apice rotundatis vel obtusissimis basi brevissime subitoque cuneatis integerrimis membranaceis, nervis 5-7-jugis tenuibus subtus prominulis, petiolo 1 cm. longo. Flores fasciculati, pedunculo 6 mm. longo, sepalis ovato-triangularibus vix 0.5 mm. longis, petalis ovatis fere 1.5 mm. longis brevissime unguiculatis, disco crasso conico sepalis altiore, staminibus filamentis complanatis brevibus, antheribus transverse ovatis transversaliter dehiscentibus filamentis vix longioribus, ovario conico, disco fere continuo et in stylum sensim attenuato, loculis 3, 2-ovulatis. Fructus obovati (7.5 cm. × 1 cm.), 2-loculares.

A n e i t y u m: Anelgauhat Bay, common in rain-forest, at sea level, no. **853**, March 4, 1929 (vine growing on rain-forest trees; leaves dark green; petals light green).

Related to *S. prinoides* DC. of Malaysia, New Guinea, Bismarck and Solomon Islands, but the leaves are different.

SAPINDACEAE

Pometia pinnata Forster, Char. Gen. 110 (1776).

T a n n a: Lenakel, common in heavy rain-forest soil at sea level, no. **19**, Feb. 21, 1928 (tree up to 15 m. high; fruit brown when ripe).—Already found on Tanna; also Fiji, Samoa, Marshall and Bismarck Islands, New Guinea and Malaysia.

Cupaniopsis aneityensis Guillaumin, sp. nov.

Arbor, ramis lepidotis, foliis 5-foliatis, jugis disjunctis, petiolo 3-4 cm. longo, foliolis lanceolatis (usque ad 8 cm. x 3 cm.) apice attenuatis basi asymmetrice cuneatis coriaceis vel chartaceis, nervis lateralibus 7-11-jugis, tenuibus, venis subtus densissime reticulatis, petiolulo 6-10 mm. longo; thyrsi ad apicem axillares, usque ad 20 cm. longi, dense lepidoti, densiflori, alabastris globosis dense lepidotis, floribus 2 mm. longis pedicello aequilongo suffultis, bracteis triangularibus minimis, sepalis 5 ovato-triangularibus extra lepidotis intus glabris, petalis minimis sepalis brevioribus cuneatis apice leviter emarginatis ciliolatisque, disco 5-glanduloso glabro, staminibus 2 mm. longis, filamentis dimidio inferiore pilosis superiore glaberrimis, antheris ovatis, germinis rudimento tereti apice rufo-penicillato.

A n e i t y u m: Anelgauhat Bay, common in rain-forest, alt. 275 m., no. **827** pro parte (TYPUS) Feb. 28, 1929 (medium-sized tree 12 m. high); same locality, common in rain-forest, alt. 100 m., no. **742** Feb. 12, 1929 (large tree, 20 m. high).

Harpullia arborea Radlkofer in Sitzb. Math.-Phys. Cl. Akad. Muench. xx. 278 (1890).

A n e i t y u m: Anelgauhat Bay, common in rain-forest, alt. 150 m., no. **939**, March 17, 1929 (large tree up to 15 m. high; petals white; calyx brown, hairy).—Already recorded from Tanna; also Samoa, Fiji and Solomon Islands and Malaysia.

Elattostachys vitiensis Radlkofer in Sitzb. Math.-Phys. Cl. Akad. Muench. IX. 602 (1879).

A n e i t y u m: Anelgauhat Bay, common in rain-forest, alt. 240 m., no. **827** in part, Feb. 28, 1929 (medium sized tree up to 12 m. high).
E r o m a n g a: Dillon Bay, common in rain-forest at sea level, no. **356**, June 3, 1928 (large tree up to 25 m. high; petals red).—Vernacular name "Ne-el-lar-ru."

ANACARDIACEAE

Spondias dulcis Forster, Fl. Ins. Austr. Prodr. 34 (1786).

E r o m a n g a: Dillon Bay, common in rain-forest at sea level, no. **311**, May 26, 1928 (large tree about 25 m. high; fruit used for jam-making).—Also Fiji, Samoa, Society and Bismarck Islands, New Guinea and Malaysia.—Vernacular name "Nevie."

Dracontomelum vitiense ? Engler in De Candolle, Monog. Phan. IV. 253 (1883).

T a n n a: Lenakel, common in rain-forest at sea level, no. **22**, Feb. 21, 1928 (tree up to 12 m. high; leaves dark green). E r o m a n g a: Dillon Bay, common in rain-forest at sea level, no. **244**, May 14, 1928 (tall tree up to 20 m.; fruit brownish yellow when ripe).—Vernacular name "Nar-ah."

Dracontomelum vitiense Engl. has been already found on Efate.

LEGUMINOSAE

Ormocarpum cochinchinense (Lour.) Merrill in Philipp. Jour. Sci. v. 76 (1910).

Ormocarpum sennoides De Candolle, Prodr. II. 315 (1825).

A n e i t y u m: Anelgauhat Bay, common along sea shore at sea level, no. **929**, March 17, 1929 (small tree up to 6 m. high; flowers white with purple veins).—Also in Australia (Queensland), New Caledonia, Bismarck Islands, New Guinea and Malaysia.

GYROCARPACEAE

Gyrocarpus americanus Jacquin, Select. Stirp. Am. Hist. 362 (1788).

E r o m a n g a: Dillon Bay, common in rain-forest at sea level, no. **371**, June 5, 1928 (large tree 25 m. high; wood soft, used for canoe-making).—Already found on the Torres Islands; also in New Caledonia, Fiji and Society Islands, Australia (Queensland, North Australia) and Malaysia.—Vernacular name "Nep-bleb-le."

MYRTACEAE

Eugenia sp.

A n e i t y u m: Anelgauhat Bay, common along sea shore at sea level, no. **787**, Feb. 20, 1929 (small tree up to 6 m. high, stunted, gnarled; leaves affected by salt water; fruit dark deep red when ripe, 2-50 in a bunch).

CORNACEAE

Alangium vitiense (A. Gray) Baillon ex Harms, Engler & Prantl, Nat. Pflanzenfam. III.-8. 262 (1898).

A n e i t y u m: Anelgauhat Bay, common in rain-forest, alt. 300 m., no. **770,** Feb. 14, 1929 (large tree up to 12 m. high; leaves dark glossy green; flowers yellow, sweetly scented).

The plant from Aneityum differs from that of the Fiji Islands and of the west coast of Australia in the much larger leaves and particularly in the filaments being nearly three times shorter than the anthers.

RUBIACEAE

Dolicholobium aneityense Guillaumin in Jour. Arnold Arb. XIII. 2 (1932).

A n e i t y u m: Anelgauhat Bay, common in rain-forest, alt. 240 m., no. **767,** Feb. 14, 1929 (plant up to 1.8 m. high; flowers white).—Already reported from Aneityum.

Psychotria sp.

B a n k s G r o u p: Vanua Lava Island, common in rain-forest, alt. 30 m., no. **413,** June 5, 1928 (small tree up to 10 m., growing in shade of larger trees; fruit red when ripe).

MYRSINACEAE

Maesa banksiana Guillaumin, sp. nov.

Arbor parva, 8 m. alta, ramis gracilibus rubris glaberrimis, foliis ovatis vel ovato-lanceolatis (circa 14 cm. x 7 cm.) membranaceis apice basique acutis integris discoloribus in sicco supra brunneis subtus viridibus, nervis 6-jugis brunneo-rubris haud vel vix prominentibus, petiolo 1.5-3 cm. longo. Inflorescentiae axillares, 2-2.5 cm. longae, racemosae, erecto-patulae, glabrae, floribus subsessilibus, bracteis lanceolatis minimis margine leviter erosis, bracteolis ovato-triangularibus margine leviter erosis 0.5 mm. longis receptaculum haud formantibus, sepalis 5 liberis triangularibus acutis margine leviter erosis 0.75 mm. longis inconspicue punctatis, petalis 5 fere usque ad medium connatis ovatis apice rotundatis fere 1 mm. longis haud punctatis nec lineolatis, staminibus petalorum tertiam supremam partem attingentibus, antheris late ovatis filamentis vix longioribus, ovario ovoideo, stylo tereti, stigmate rotundato.

B a n k s G r o u p: Vanua Lava Island, common in rain-forest, alt. 300 m., no. **456,** July 12, 1928 (small tree up to 8 m. high).

In the key given by Mez (in Engler, Pflanzenr. IV.-236, p. 23) this new species should be placed beside *M. denticulata* Mez of the Philippine Islands.

OLEACEAE

Ligustrum neo-ebudicum Guillaumin, sp. nov.

Arbor magna, 20 m. alta, ramis pallide fulvis rugose lenticellatis,

foliis ovatis (usque ad 10 cm. x 4 cm.) basi cuneatis apice longe saepeque falcatim acuminatis subcoriaceis, nervis 8-10-jugis tenuibus, venis immersis, petiolo circa 1.5 cm. longo. Paniculae ad apicem axillares, ad 5 cm. longae, minute puberulae, floribus pedicello brevi (0.5 mm.) basi bractea 1 apice 2 ad apicem minute puberulis munito, calyce dentibus 4 triangularibus acutis margine minutissime puberulis, petalis albis 1.5 mm. longis ovatis quarta inferiore parte connatis, staminibus filamentis brevissimis corollae tubo vix aequilongis, antheris ovato-linearibus apice truncato rotundatis nigroque punctatis, ovario ovoideo minimo stylo aequilongo, stigmate capitato.

A n e i t y u m: Anelgauhat Bay, alt. 300 m., rain-forest, common, no. **762,** Feb. 14, 1929 (large tree up to 18 m. high; petals white).

Near *L. novo-guineense* Lingelsh. of New Guinea, but differs in the calyx and particularly in its stamens with very short filaments and not apiculate anthers.

Linociera ? ramiflora Wallich, Cat. 2824 (1831), nomen.—De Candolle, Prodr. VIII. 297 (1844).

A n e i t y u m: Anelgauhat Bay, common in rain-forest, alt. 300 m., no. **765,** Feb. 14, 1929 (large tree up to 18 m. high; leaves dark glossy green; petals white); same locality, alt. 210 m., no. **823,** Feb. 28, 1929 (leaves light yellow-green; petals pink-white).

The plant of Aneityum differs from that of New Guinea, Queensland and Malaysia in the petals which elongate after the opening of the flower and attain finally a length of 5 mm. before dropping.

MYRISTICACEAE

Myristica inutilis Richard apud A. Gray, Bot. U. S. Expl. Exped. I. 34 (1854).

A n e i t y u m: Anelgauhat Bay, common in rain-forest, alt. 60 m., no. **757,** Feb. 13, 1929 (large tree up to 18 m. high; leaves golden brown below; fruit golden brown). T a n n a: Lenakel, common in rich rain-forest soil, alt. 60 m., no. **44,** Feb. 24, 1928 (tree 15 m. high, 25 cm. in diam.; leaves brown below). E f a t e: Undine Bay, common in rain-forest, alt. 60 m., no. **220,** April 27, 1928 (tree 12-15 m. high; leaves brown below; fruit brown).—Also in the Samoa and Tonga Islands.

LAURACEAE

Cryptocarya tannaensis Guillaumin, sp. nov.

Arbor ultra 15 m. alta, cortice brunneo griseo-maculato, ramis primum rufo-puberulis deinde glabris in sicco nigris, foliis ovatis (usque ad 10 cm. x 5 cm.) apice acutis basi cuneatis leviter rigidis, glaberrimis, nervis lateralibus 5, venis densissime reticulatis, petiolo circa 1

cm. longo. Paniculae 2 cm. longae, axillares terminalesve, ramis pedicellisque rufo-puberulis; flores pedicello brevissimo (1 mm.) apice bracteolato, perianthii tubo campanulato lobis fere aequilongo extra puberulo intus glabro, lobis 6 ovatis extra puberulis intus densius pilosis, staminibus generis, filamentis breviter ut connectivo hirsutis, antheris ovato-triangularibus obtusis filamentis vix longioribus, staminodiis brevius stipitatis acutis, ovario elongato glabro, stylo tereti antherarum apicem attingente.

T a n n a: Lenakel, common in rich rain-forest at sea-level, no. **18**, Feb. 21, 1928 (tree up to 15 m. high; bark brown with round grey spots).

Litsea ? sp.

T a n n a: Lenakel, rain-forest, not common, alt. 30 m., no. **140**, March 8, 1928 (small tree about 7 m. high; flowers white).

EUPHORBIACEAE

Glochidion sp. aff. *G. manono* Baill. of the Fiji and Society Islands.

A n e i t y u m: Anelgauhat Bay, common in the country, in poor red soil, alt. 15 m., no. **700**, Feb. 4, 1929 (small tree up to 8 m. high; leaves light yellow-green; petals yellow).

Baccaurea sp.

A n e i t y u m: Anelgauhat Bay, common in rain-forest, alt. 60 m., no. **752**, Feb. 12, 1929 (large tree up to 12 m. high; fruit red when ripe).

Baccaurea sp.

A n e i t y u m: Anelgauhat Bay, common in rain-forest, alt. 240 m., no. **772**, Feb. 14, 1929 (large tree up to 15 m. high; leaves dark green).

Baccaurea ?

T a n n a: Mt. Tokosh Meru, common in rain-forest at 600 m., no. **168**, March 15, 1928 (tree about 15 m. high).

Aleurites moluccana Willdenow, Sp. Pl. IV. 590 (1805).

A n e i t y u m: Aname, common at sea side, alt. 15 m., no. **989** (coll. *J. P. Wilson*), Sept. 1929 (large tree 1.3 m. in diam.; fruit brown).—Already found on Efate; also New Caledonia, Loyalty, Fiji, Tonga, Samoa, Cook, Society, Marquesas, Gambier and Mariana Islands, New Zealand, Australia (Queensland), New Guinea, Malaysia and Hawaii.—Vernacular name "Inhatch."

MORACEAE

Paratrophis tahitensis (Bur.) Drake, Ill. Fl. Ins. Mar. Pacific. 296 (1892); Fl. Polyn. Franç. 193 (1892).[1]

A n e i t y u m: Anelgauhat Bay, common in rain-forest, alt. 210 m., no. **818,** Feb. 28, 1929 (large tree up to 12 m. high; flowers white; leaves boiled and eaten by natives).

URTICACEAE

Leucosyke corymbulosa Weddell in De Candolle, Prodr. XVI[1]. 235[30] (1869).

A n e i t y u m: Anelgauhat Bay, common in rain-forest at sea level, no. **791,** Feb. 20, 1929 (small tree up to 9 m. high; fruit white when ripe, with green seeds).

UNDETERMINED NUMBERS

The following numbers have not been identified owing to insufficient material: nos. 159, 166, 297, 379, 750, 807, 808, 821 and 841 (= 166).

MUSEUM NATIONAL D'HISTOIRE NATURELLE,
PARIS

[1]This name is usually attributed to Bentham & Hooker (Gen. Pl. III. 364), but these authors have only identified the genus *Uromorus* Bur. with *Paratrophis* and did not make the binomial combination.

A SUPPLEMENT TO C. T. WHITE, "LIGNEOUS PLANTS COLLECTED IN THE TERRITORY OF PAPUA (BRITISH NEW GUINEA) IN 1925-26 BY L. J. BRASS"[1]

ALFRED REHDER

PALMAE

Determined by M. BURRET

Calamus nannostachys Burret in Jour. Arnold Arb. XII. 264 (1931).

Kuraudi, Eastern Division, no. **1379**.

Areca (Balanocarpus) **nannospadix** Burret, l. c. 265.

Ihu, Vailalo River, rain-forest, no. **921**.

Actinophloeus microcarpus Burret, l. c. 267.

Loloki River, in dumps on river bank, no. **1659**.

Actinophloeus linearis Burret, l. c. 268.

Lower Mori River, Eastern Division, rain-forest, no. **1566**.

MORACEAE

Ficus

Determined by V. S. SUMMERHAYES

In my account[2] of the Figs collected in Papua by L. J. Brass on behalf of the Arnold Arboretum I inadvertently used for two new species of *Ficus* names which had previously been used for quite other species. It therefore becomes necessary to rename the Papuan species; these are as follows:

Ficus aechmophylla Summerhayes, nom. nov.

Ficus Brassii Summerhayes in Jour. Arnold Arb. x. 146, 207 (1929); non R. Br.

Loloki River, no. **1660**.

Ficus skytinodermis Summerhayes, nom. nov.

Ficus clusiacfolia Summerhayes l. c. 152, 209; non Schott.

Sogeri, foothill forest, alt. 500 m., no. **641**.

Ficus Decaisneana Miquel, Fl. Ind. Bat. I. pt. II. 312 (1859).

Loloki River, no. **549**, and Bongwina River, Eastern Division, no. **1620**; cited (l. c. 206) under *Ficus subulata* Bl.

Conocephalus sp.

Lowa, Vailala River, alt. 60 m., on rain-forest trees, no. **1146**, March 15, 1926 (climbing; attaching itself to trunks of trees by adventive roots; flowers axillary, pink).

[1]See Jour. Arnold Arb. x. 197-274 (1929) for the original paper. The determinations in this supplement were mostly received from the Botanical Museum, Berlin-Dahlem.

[2]Jour. Arnold Arb. x. 142-154 (1929).

MYRISTICACEAE

Myristica sp.

Bisiatabu, alt. 300 m., no. **610,** Nov. 11, 1925 (compact tree, 10 m. high; bark channelled.

LEGUMINOSAE

Pithecolobium sp. aff. *P. umbellatum* Benth.

Keuru, Gulf Division, sea beach, no. **1197,** March 22, 1926 (small tree, 5 m. high; leaves dark bluish green; stamens pink.)

Mucuna pruriens De Candolle, Prodr. II. 405 (1825).

Domara River, Eastern Division, no. **1594,** June 31, 1926, (large climber; flowers purple; fruit silky-hairy).

Pueraria novoguineensis Warburg in Bot. Jahrb. XIII. 325 (1891).

Ihu, Vailala River, no. **957,** Feb. 12, 1926 (climbing over bushes on river bank; flowers large, blue).

CELASTRACEAE

Siphonodon sp.

Ihu, Vailala River, rain-forest, no. **1074,** Feb. 26, 1926 (small slender tree; fruit clusters on trunk).

SAPINDACEAE[1]

Determined by H. MELCHIOR

Allophylus micrococcus Radlkofer in Schumann & Lauterbach, Fl. Deutsch. Schutzgeb. Südsee Nachtr. 307 (1905).

Ihu, Vailala River, rain-forests, no. **950,** Feb. 12, 1926 (slender tree, 12-15 m., with thin gray bark; fruit yellow); Hewa, Vailala River, rain-forests, no. **1132,** March 13, 1926 (small tree with close gray bark; fruit bright red); Kuraudi, Eastern Division, on river bank, no. **1385,** May 12, 1926 (small tree).—Common name "Odia" (no. 1132) and "Pidi-Pidia" (no. 1385).—See also Jour. Arnold Arb. x. 235.

Alectryon ferrugineus Radlkofer in Act. Congr. Bot. Amsterd. 1877, p. 84 (1879).

U-uma River, on river bank, Eastern Division, alt. 300 m., no. **1522,** May 20, 1926 (spreading tree 10 m. high; seeds glossy black, partly enclosed in a pink arillus).

Alectryon reticulatus Radlkofer in Sitzb. Math.-Phys. Cl. Akad. Muench. xx. 255 (1890).

Bomgwina River, Eastern Division, no. **1618,** *June* 2, 1926 (small river bank tree; flowers pink, in axillary panicles).

Guioa rigidiuscula Radlkofer in Sitzb. Math.-Phys. Cl. Akad. Muench. IX. 614 (1879).

[1]See also Jour. Arnold Arb. x. 235 (1929).

Bisiatabu, alt. 450 m., no. **594,** Nov. 8, 1925 (slender tree, 10 m. high, with thin pale gray bark; fruit rufous).—Common name "Ete."

Guioa spec., aff. *G. aryterifolia* Radlkofer.

Bisiatabu, edge of rain-forest, alt. 450 m., no. **614,** Nov. 12, 1925 (tree 10 m. high, with thin gray bark; fruits brown in clustered panicles on main branches).

Cupaniopsis spec.

Iawarere, rain-forest, alt. 300 m., no. **706,** Nov. 29, 1925 (slender unbranched tree, 3 m. high, with a crown of long spreading pinnate leaves; fruits orange-tinted in axillary racemes or panicles).—Common name "Sisimana."

Elattostachys tetraporandra Radlkofer in Sitzb. Math. Phys. Cl. Akad. Muench. xx. 267 (1890).

Kapa Kapa, tidal flats, no. **795,** Dec. 7, 1925 (spreading tree to 7.5 m. high, with brown close bark); Rigo, coastal brush, no. **815,** Dec. 9, 1925 (spreading tree 7.5 m, high, with rough gray bark).

Elattostachys spec.

Domara River, rain-forests, Eastern Division, no. **1601,** May 31, 1926 (tree 9-12 m. high; fruit red, seeds black).

Lepidopetalum hebecladum Radlkofer in Sitzb. Math.-Phys. Cl. Akad. Muench. xx. 269 (1890).

Mowabula, Eastern Division, no. **1371,** May 10, 1926 (small river-bank tree; fruit red).

Dodonaea viscosa Jacquin, Enum. Pl. Carib. 19 (1760).

Bomgwina, Eastern Division, no. **1612,** June 1, 1926 (small beach tree).—See also Jour. Arnold Arb. x. 236.

Harpullia camptoneura Radlkofer in Sitzb. Math.-Phys. Cl. Akad. Muench. xx. 360 (1890).

Kapa Kapa, tidal flats, no. **806,** Dec. 8, 1925 (erect tree, 3 m. high; fruit yellow).

Harpullia cupanioides Roxburgh, Hort. Beng. 86 (1814), nomen; Fl. Ind. L. 645 (1832).

Kerema, Gulf Division, rain-forests, no. **1215,** March 24, 1927 (slender tree 3-4.5 m. high; flowers urceolate, white, in axillary panicles); Kuraudi, Eastern Division, common in riverine rain-forests over a large area of the south coast, no. **1394,** May 12, 1926 (large bush or sparsely branched slender tree; flowers greenish in axillary or supra-axillary panicles); U-uma River headwaters, on river bottom, alt. 300-450 m., Eastern Division, no. **1450,** May 18, 1926 (large sparsely branched bush or small tree, 2.5 m. high; flowers white, sweet-scented in axillary panicles); lower Mori River, Eastern Division, riverine rain-forests, no. **1558,** May 28, 1926 (slender small tree or bush 3-4.5 m. high;

flowers greenish white; fruit scarlet, seeds yellow).—Common name "Gari" (no. 1558).

Alphitonia moluccana Teysmann & Binnendijk, Cat. Hort. Bogor, 221 (1866), nomen.—Braid in Kew Bull. Misc. Inform. 1925, p. 184.

Alphitonia zizyphoides C. T. White in Jour. Arnold Arb. x. 236 (1929); not A. Gray.

Nos. **1497** and **1637** (see White, l. c.).

According to K. W. Braid in his Revision of the genus *Alphitonia* (l. c.), *A. moluccana* is not identical with *A. zizyphoides* which is restricted to Polynesia.

RUBIACEAE

Psychotria polyneura Val. vel aff.

Ihu, Vailala River, rain-forest, no. **962**, Feb. 13, 1926 (small soft-wooded bush, leaves somewhat fleshy).

COMPOSITAE

Microglossa volubilis De Candolle, Prodr. v. 320 (1836).

Uniori, alt. 250-300 m., light rain-forest, no. **739**, Dec. 2, 1925 (soft rambling under shrub).

APPENDIX
LIST OF HERBACEOUS PLANTS COLLECTED IN NEW GUINEA BY L. J. BRASS

CYPERACEAE

Remirea maritima Aubl.
No. 1614

Mapania macrocephala (Gaudich.) K. Schum.
No. 1158

ARACEAE

Rhaphidophera novo-guineensis Engl.
No. 1034 (juvenile stage).

Cyrtosperma Merkusii (Hassk.) Schott
No. 931

Schismatoglottis calyptrata (Roxb.) Zoll. & Moritzi
No. 916

Alocasia lancifolia Engl.
No. 913

COMMELINACEAE

Pollia macrophylla Benth.
No. 652

Aneilema sp.
No. 999

Forrestia hispida Less. & Rich.
No. 1124

LILIACEAE

Dianella ensifolia Red.
No. 1430

AMARYLLIDACEAE

Crinum macranthum Engl.
No. 1157

TACCACEAE

Tacca pinnatifida Forst.
No. 1169

DIOSCOREACEAE

Dioscorea bulbifera L.
No. 1030

Dioscorea tiliifolia Kunth
No. 867

MUSACEAE

Musa sp.
No. 1364

ZINGIBERACEAE

Curcuma longa L.
No. 548

Tapeinochilus sp.
No. 608

Costus speciosus (Koenig) Smith
No. 922

Alpinia novae-pommeraniae K. Schum.
No. 1373

MARANTACEAE

Donax canniformis (Forst.) Rolfe
No. 926

Phrynium macrocephalum K. Schum.
No. 960 in part

Phrynium pedunculatum Warb.
No. 564, 960 in part

ORCHIDACEAE

Agnostophyllum superpositum Schlechter
No. 1493

Appendicula cleistogama Schlechter
No. 1216

Oberonia diura Schlechter
No. 1523 (not no. 1216 as cited in Jour. Arnold Arb. x. 273).

AMARANTACEAE

Deeringia amaranthoides (Lam.) Merr.
No. 869

CERATOPHYLLACEAE

Ceratophyllum sp. an *C. demersum* L.
No. 1621

BALSAMINACEAE

Impatiens Hawkeri W. Bull.
No. 554

BEGONIACEAE

Begonia sp.
No. 919

CONVOLVULACEAE

Calonyction bona-nox (L.) Boj.
No. 857

Ipomoea denticulata (Desv.) Choisy
Nos. 1029, 1639

Ipomoea sp.
No. 1095

Jaquemontia paniculata (Burm.) Hallier f.
No. 1404

LABIATAE

Dysophylla auricularia Bl.
No. 1122.

Coleus scutellarioides Benth.
No. 767.

Orthosiphon stamineus Bl.
No. 833.

SOLANACEAE

Solanum sp.
No. 972

SCROPHULARIACEAE

Limnophila rugosa (Rostk.) Schlechter
No. 1651

Ilysanthes veronicifolia (Retz.) Urb.
No. 1014

GESNERIACEAE

Cyrtandra sp. aff. *C. bracteata* Warb.
No. 1453

ACANTHACEAE

Strobilanthes sp. aff. *S. novo-megapolitanus* Lindau
No. 1082

RUBIACEAE

Nertera depressa Banks & Sol. var. **papuana** Val.
No. 1445

COMPOSITAE

Vernonia cinerea (L.) Less.
No. 752

Vernonia lanceolata (Warb.) Moq.
No. 740

Adenostemma viscosum Forst.
No. 1036

Blumea Lessingii Merr.
No. 756

Blumea pubigera (L.) Merr.
No. 1363

Eclipta alba (L.) Hassk.
No. 893

Wedelia biflora (L.) DC.
Nos. 719, 958, 1382

Wedelia spilanthoides F. v. Muell.
No. 775.

Herbarium, Arnold Arboretum
Harvard University

INHERITANCE IN AN OAK SPECIES HYBRID

S. H. YARNELL

With eight text figures

A report of the first generation of the cross (*Quercus virginiana* (Live Oak) × (*Q. lyrata* (Overcup Oak)[1] was made by the late Helge Ness (1918) who, a few years later, was successful in obtaining three second generation families totaling twenty-three plants. While Professor Ness raised this second generation to fruiting maturity, he never made a second report. This paper presents a study of segregation of characters in the above F_2.

The original cross was made in 1909 using the Live Oak as female; this was duplicated the following year. This species cross is easily made in spite of the fact that the parents belong to extremes of the section Lepidobalanus. Two of the four F_1 trees planted in front of the Station buildings are still growing vigorously and correspond very closely to the early description. They show definite evidence of hybrid vigor. They are now 13.4 and 14.8 inches in diameter at four and one-half feet above the ground. Eleven Live Oaks planted three years later average only 5.8 inches in diameter at the same height. Twenty Overcup Oaks planted with the Live Oaks average 5.97 inches. While the F_1 hybrids are perhaps a little more advantageously situated than the others, their diameter, which is more than twice that of plants of either parental species, can not be accounted for either by their situation or by their three-year start. All F_1 plants of this cross were especially vigorous growers as seedlings.

The 23 second generation hybrids belong to three families as follows: five trees from F_1 parent No. 1, ten from parent No. 2 and nine plants from parent No. 5. F_1 parent No. 1 resulted from the 1909 cross; parents No. 2 and No. 5 from the later cross. Since the F_1 plants are quite uniform and since the numbers in the second generation are small, all F_2 plants have been considered together as a single family. Seed to produce the second generation was collected in 1919, the trees being set out in 1923, spaced ten feet apart each way.

[1] It is an interesting fact that trees supposed to be natural hybrids between the Live Oak (*Quercus virginiana*) and the Overcup Oak have been found in several places along the Gulf coast. Specimens from a number of trees found in the vicinity of Natchez, Mississippi, by Miss C. C. Compton were sent to the Arnold Arboretum in 1915 and 1916, and Professor Sargent published a description of them under the name *Quercus Comptonae* in the Botanical Gazette, LXV. 456-458 (1918).

The earliest discovery of the tree, however, seems to have been by Dr. Chas. H.

LEAF CHARACTERS

The leaves of the Overcup Oak are approximately twice as long as those of the Live Oak. While the leaves of the F_1 plants are large, they are smaller than those of the male parent. A random sample of 50 mature leaves were secured from each second generation tree and from representatives of the parental species. Measurements were taken, in centimeters, of the length and greatest width. The means of these two dimensions were added to serve as an arbitrary index of leaf-size. In relation to leaf-area this method exaggerates the size of the *lyrata*-type leaf. The results are presented in Figure 1. The F_2 plants fall into

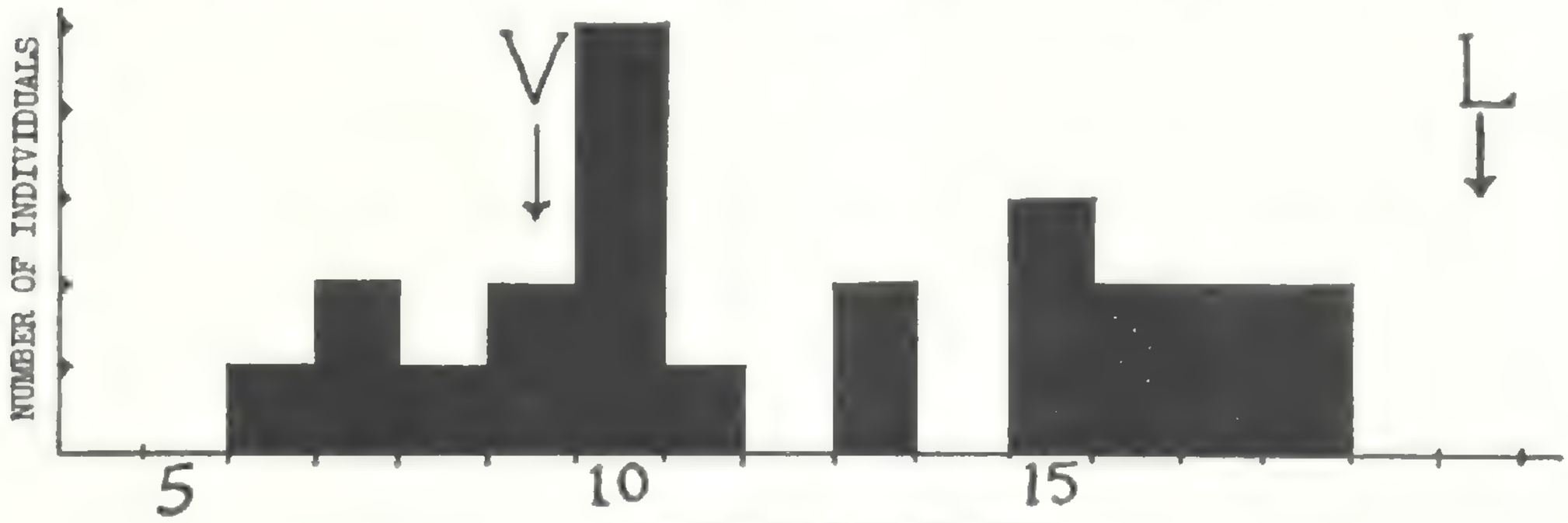

FIGURE 1. SEGREGATION OF LEAF SIZE IN THE F_2.
The indices of the parental species are marked by arrows.

two distinct groups, one ranging around the *virginiana* parent, the other in a distribution below the *lyrata* parent. The curve of the first group shows a decided tendency to flatten out, the other group forms approximately a straight line. The two groups are about equal in size, there being 12 individuals in the first and 11 in the second. This occurrence

Mohr, along Paysons Creek, Matagorda Co., Texas. A specimen of his collection, No. 96, Dec. 18, 1880, is now in the herbarium of the Arnold Arboretum. The label on this specimen also bears the notation: "A large low wide-spreading tree, with persistent foliage." The foliage is of the "small-leaf" group of Dr. Yarnell. The tree seems to have disappeared from this locality, or at least a diligent search for it by the writer in 1916 failed to find any trace of it.

It is not stated on Dr. Mohr's label whether the tree found by him was native or planted, but in the absence of such notation, it may be assumed that it was wild. With the exception of this and of two seedlings found in the woods near Natchez, all of the trees known seem to have been planted either in parks, as street trees, or about dwellings and plantations. But no records were obtained as to where they came from, except in the case of some trees in New Orleans, which were reported to have been brought from across Lake Pontchartrain 30 or 40 years previously.

Miss Compton, as stated by Sargent, located some 20 or 30 trees of this hybrid growing in the vicinity of Natchez, and specimens in the herbarium here show it to have been collected near Selma, Alabama, and along the streets and in Audubon Park, New Orleans. These specimens show a wide variation in leaf and fruit characters, with many of them intermediate between the two parents, and similar to those described by Dr. Yarnell for the Ness hybrid and its descendants.

The rather wide natural distribution would seem to indicate that the two species, in spite of their obvious morphological differences, hybridize readily when growing together.

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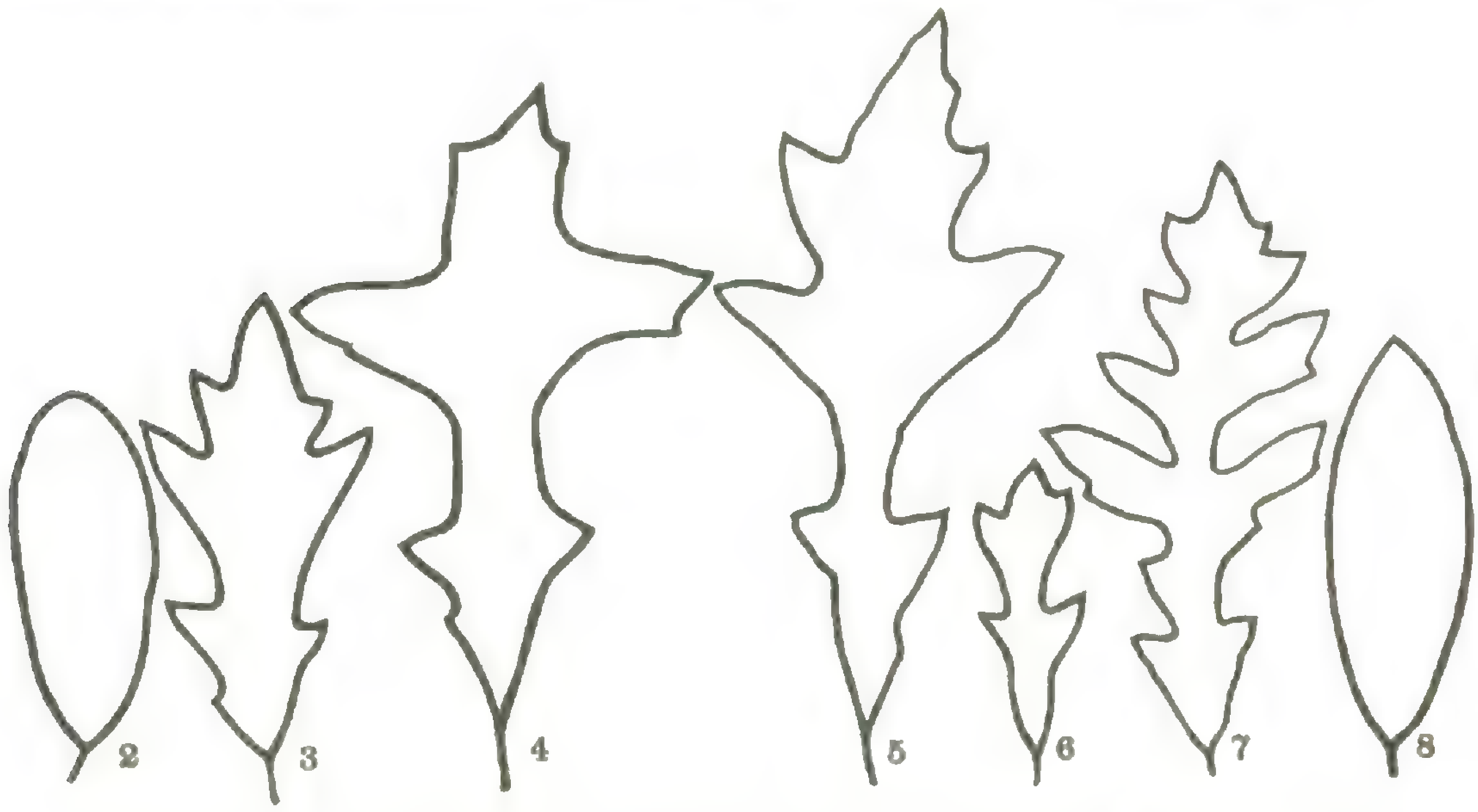
of two distinct groups of approximately equal size has been interpreted as evidence that two principal factors are involved in size of leaf and that the distribution obtained represents a 9:7 ratio, the lack of agreement being due to small numbers.

Since segregation is taking place in the second generation of a rather wide interspecific cross, it is reasonable to assume that a number of factors from one species influence the expression of characters normally found in the other. Such an influence is evident in the expression of each of the characters under study, but does not, it is believed, invali-



FIGURES 2–8. PARENTAL, F_1 AND F_2, LEAF SHAPE TYPES, × ¾.
2. QUERCUS VIRGINIANA. 3. THE F_1. 4. Q. LYRATA.
5–8. F_2 PLANTS.

date an estimate of the number of principal factors involved. This, together with a lack of complete dominance, accounts for the amount of variation found in the second generation.

Perhaps the most difficult classification to make, on this account, is that of leaf shape. The male parental species derives its name from the lyrate character of its foliage. The leaf of the female parent, while for the most part entire, does show a tendency toward the production of slight irregularities on some of the leaves, producing a type of margin which can not be distinguished, except in degree, from certain of the F_2 segregates. The *virginiana* type may be seen in Figure 2, the *lyrata* type in Figure 4. The F_1 is outlined in Figure 3. The figures have been traced from representative leaves and have been reduced ¼ in reproduction. Figures 5 and 8 illustrate extreme types in the F_2. The leaf in Figure 5 is the closest approach to the *lyrata* type. The leaf in Figure 8 is *virginiana*-like except for the apex which is pointed. There is one such plant. Three others have leaves very close to those of the

female parent, with the addition of small lobes. Seven others are considered nearer this type. All of these are in the "small-leaf" group discussed above. The leaf in Figure 6 illustrates the only plant in the "small-leaf" group which is distinctly lyrate in shape. All trees in the "large-leaf" group have lyrate leaves. The exact form in some cases varies rather widely from the *lyrata* type as may be seen in Figure 7. This is presumably due to this influence of incidental factors from the Live Oak parent. The fact that one recessive was recovered in the F_2 and that all such plants can be placed in two general categories is good evidence that not more than two principal factors are involved in determining leaf shape. Since the grouping with respect to size and shape of leaves coincide with one exception, the relationship of the two groups of factors can not be determined. A number of things might be responsible, such as identity or close linkage of factors, gametic selection, or inviability of certain gametic or zygotic combinations.

The epidermis of the Live Oak is somewhat thickened, giving the leaves a shiny appearance, in contrast to the duller and thinner leaves of the Overcup Oak. The coriaceous character is not completely dominant in the F_1, heterozygous plants being classified as modified coriaceous. The leaves of all plants in the "small-leaf" group were coriaceous. In addition, the leaves of three trees in the "large-leaf" group were given the same classification. This gives a total of 15 coriaceous and 8 non-coriaceous plants. This is a little nearer the 9:7 than to the 3:1 expectancy (13:10 and 17.25:5.75 respectively).

A related character is the habit of dropping or retaining the leaves at the end of the summer season. The name "Live Oak" is due to the evergreen character of *Q. virginiana*. The pollen parent, on the other hand, sheds its leaves relatively early. The first generation hybrid holds only a part of its leaves during the winter. All of the trees with large, non-coriaceous leaves are completely deciduous. In addition, two of the three trees with large modified coriaceous leaves were fully deciduous, while the third was classified as deciduous with a tendency to hold a part of its leaves later in the season. Five of the twelve trees with "small" coriaceous leaves were completely evergreen, the remainder being partially evergreen. It is evident that not more than two principal factors are involved here and quite possibly only one pair.

FRUIT CHARACTERS

Fruits of the two parental species differ widely in appearance, especially in regard to size, shape and character of the cupule. Twelve of the twenty-three second generation trees fruited during the 1931 season.

Fruits of *Q. virginiana* are approximately twice their diameter in

length while those of *Q. lyrata* are somewhat wider than long. In the F_1 the fruits are wider in proportion to length than those of the female parent. Acorns of eight trees are distinctly long, of two are intermediate, and of two decidedly round, giving a total of 8 long to 4 round. Inheritance of fruit shape seems to be similar to that of leaf shape with the former giving more nearly complete dominance. The fact that no fruits precisely like those of the *lyrata* parent have been found lends support to a two-factor hypothesis.

There is a striking difference between the two species in size of fruit, the larger diameter of *lyrata* fruit giving the greater volume. Acorns of the first generation usually exceed those of either parent in size since they are somewhat longer than those of *virginiana* and nearly as wide as in *lyrata*. In every case in the F_2 the fruits have been classified as small and in three individuals the fruit is considerably smaller than in the *virginiana* parent. How much of this may be due to irregularities of development resulting from the interspecific nature of the cross can not be said. It is clear that the results can not be explained on a simple factorial basis.

The Overcup Oak is so named because the cupule covers from three-fourths to nearly the entire nut. In the Live Oak fully three-fourths of the acorn is exposed. The cupule of the first generation is intermediate in size, covering from one-third to one-half of the large acorn. In nine F_2 plants the cupule is about as found in the female parent. In two trees the cupule is intermediate in size, and in one the cupule approaches but does not equal that of the pollen parent in size. While two factors are indicated in this case it is evident that the results are complicated by other factors such as size of fruit.

Thickness of cupule scales is a related character. While the scales are nearly equal in number in the two species they are much enlarged in the Overcup Oak and have a protuberance at the tip. The cupule scales in the F_1 may be said to be intermediate in size, although they are much closer to those of the Live Oak parent. In the F_2 eight individuals have thin scales and five have scales intermediate in thickness. None has scales approaching those of the Overcup Oak in prominence. Since this character shows such a close relationship with the preceding it is likely that the two have one factor in common.

BARK CHARACTER

It has been possible to obtain data in regard to one other character, type of bark. In *Q. lyrata* the bark flakes off, while in *Q. virginiana* the bark of the trunk is rough. The first generation trees are intermediate in this respect. In the second generation 7 trees have bark exhibiting different degrees of flakiness while 16 trees have a rough

trunk. This ratio is probably the result of segregation of a single factor pair. This character, in common with the others under consideration, exhibits considerable variability in the F_2.

DISCUSSION

Augustine Henry (1910) studied large numbers of seedlings (971) of what was very likely the second generation of a natural cross between *Ulmus foliacea* and *U. glabra*. The F_1 had received the name *U. vegeta*, known popularly as the Huntingdon Elm. Seedlings of both parental species were quite uniform. Three characters, opposite vs. alternate leaves, leaf size, and length of petiole, were found to segregate on a 3:1 basis in the second generation. Different F_2 trees were found to vary greatly in degree of fertility.

Plate 24 of the paper cited above illustrates the Lucombe Oak, parents and seedlings. The leaf size and shape combinations in the F_2 are very similar to those of the Live × Overcup Oak cross. The Lucombe is evidently a seedling of a *Q. cerris* (Turkey Oak) × *Q. suber* (Cork Oak) cross. It is subevergreen and was propagated by grafting at Exeter, England in 1763. In 1792 acorns of this Oak were sown which gave a variable progeny (Henry 1910). The author recognizes hybrid vigor in the first generation of these and other tree crosses and the resulting increase in usefulness, and later made numerous controlled crosses with this end in view.

H. A. Allard (1932) has made a study of a number of natural Oak hybrids growing in or near the District of Columbia. Open pollinated seed of *Q. Saulii*, considered to be a hybrid between *Q. alba* and *Q. montana*, were planted about 1922. Forty seedlings grew, most of which survived. Characters of *Q. montana* are more evident in the "F_2" than those of *Q. alba*. The author points out that the generation involved is necessarily unknown. No factorial analysis was attempted. He reports that D. T. McDougal (1907) raised 55 seedlings of Bartram's Oak (*Q. heterophylla*), a natural hybrid between *Q. phellos* and *Q. borealis maxima*. Plants similar to each parent and various types of intermediates were obtained.

The first generation of the Live and Overcup Oak cross shares with other interspecific tree crosses the characteristic of rapid growth. In habit of growth it is intermediate between its two parents, being more erect than the Live Oak and having a greater spread than the Overcup parent. As a result, it is much more attractive as an ornamental than the latter species. Growth of the second generation trees varies widely, from somewhat better than the parental species to individuals that are decidedly stunted.

The tendency for characters of one parent to be inherited together in the F_2 is striking. It is a problem of importance to the plant breeder who makes use of interspecific crosses. Normal chromosomal linkage would account in some measure for this result, especially since it is likely that there is less crossing over in the F_1 than in either pure species. The partial sterility found in the second generation is good evidence that the chromosomal complements of the two species are sufficiently differentiated to prevent their normal functioning in certain combinations. It is but a step to assume that other combinations are unable to produce a viable individual. The chance for securing the desired grouping of characters under such conditions will depend upon the size of F_2 or back cross populations, which present an opportunity for viable chromosome combinations carrying the desired characters.

The results suggest, for example, that the evergreen character might be combined with hardiness to extend the range of the Live Oak type considerably north of Virginia. This would involve a number of genes from each species such as those for thickened epidermis, leaf-size, and also for physiological characters. Such a successful combination, while not common in the F_2 would be expected to occur frequently enough to justify a systematic search for such individuals.

There is ample evidence that the normal expression of a character is frequently modified by new combinations of genes from the two species. The idea that a gene may influence the expression of characters which are determined primarily by other genes has been frequently advanced. This idea usually carries with it the assumption that either allelomorph has the same incidental effect regardless of differences in the morphological expression of the allelomorphic pair. The fertile species cross with its high variability of character expression in the second generation bears out the above assumptions, in that what are presumably allelomorphic genes in the two species seem to have different incidental effects in contrast to the situation for an allelomorphic pair within a single species.

The low number of principal factors involved for many characters increases the chance of securing a satisfactory combination in the second generation. Inheritance in forest trees thus appears to be no more complex than inheritance in most material of interest to the plant breeder.

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TAXONOMIC AND CYTOLOGICAL RELATIONSHIPS OF YUCCA AND AGAVE

SUSAN DELANO MCKELVEY AND KARL SAX

With plate 55

The senior author, during the past two years, has made an extensive study of *Yucca* and related genera. These genera have been collected from western Texas, New Mexico, Arizona, and the southern areas in California, Nevada, Utah and Colorado. The distance covered in this area has approximated 25,000 miles, and no day of travel passed without encountering numerous species of these plants.

Identification of *Yucca* species in the field has been difficult because of the extreme variability of all species, and the overlapping range of two or more similar species. For example, in the group of filiferous-leaved Yuccas with dehiscent fruit, the leaf characters have at times been used as a basis for species differentiation, but as a practical guide in the field these characters are often unreliable and confusing. On a single inflorescence the flowers vary little in size and form, but two inflorescences from adjoining plants, apparently of the same species, may differ in habit; the flowers may differ in shape and size of corolla-segments, ovary and style; and vary in length, form and pubescence of filaments. Among the fleshy-fruited Yuccas, one species seems to have two distinct forms; one with a vigorous fleshy inflorescence and large flowers, and the other with a compact, more ligneous inflorescence and more numerous small flowers.

In regions where closely allied species occur, their separation in the field is extremely difficult. Within the range attributed to *Y. angustissima* are plants of vigorous growth quite different than the expected type. In some respects, they resemble *Y. glauca* whose range is supposed to begin hundreds of miles further east. At the point of worst confusion has one encountered intermediate segregates from species hybrids, or are different types variants of a single species?

In most respects the allied genera *Clistoyucca, Hesperoyucca* and *Samuela,* separated by Trelease (1902), are similar to *Yucca. Yucca* and *Agave* are also similar in various striking details although these genera are placed in different families by most taxonomists.

The occurrence of transitional forms of *Yucca* species and the similarity of closely, and even distantly related genera, has led to a cytological study of these plants in order to compare their chromosome number and morphology. Material was collected in the field by the senior

author. Flower buds at different stages of development were fixed in a mixture of acetic acid and alcohol, and later transferred to 70 percent alcohol. Chromosome counts were obtained from aceto-carmine smears by the junior author.

Permanent smears were also made from pollen mother cells of *Agave americana* collected at the Bermuda Biological Station, from *Agave virginica* from the Missouri Botanical Garden,, and from *Yucca flaccida* and *Y. filamentosa* grown in the Arnold Arboretum.

The chromosomes of *Yucca flaccida* have been described in detail by O'Mara (1931). At the first meiotic division there are 5 pairs of long chromosomes and 25 pairs of very small chromosomes. The long chromosomes have an average chiasma frequency of 3 per bivalent at metaphase, while each small bivalent has only a single terminal chiasma. The pairing is very regular and lagging univalents or other abnormalities were not observed. The extreme differences in chromosome size and the large number of chromosomes is rather unusual in the plant kingdom.

All of the species of *Yucca* examined have the same chromosome constitution. These include *Y. flaccida, Y. filamentosa, Y. elata, Y. constricta, Y. rupicola, Y. macrocarpa,* and *Y. angustissima.* The chromosomes of *Y. flaccida* and *Y. filamentosa* are shown at different stages of meiosis (figures 1, 2, and 3). The five pairs of large chromosomes are conspicuous at all stages. There is considerable variation in the size of the 25 small chromosomes, but all are relatively small.

The closely related genera examined also have exactly the same chromosome constitution as *Yucca.* These include *Hesperoyucca Whipplei, Hesperaloe parviflora,* and *Samuela Faxoniana.* Unfortunately, favorable material was not obtained from the related genera *Nolina* and *Dasylirion.* Counts from somatic cells of *Nolina* show that there are about 38 chromosomes which differ considerably in size, and most, if not all, of those chromosomes have median or sub-median spindle fiber constrictions. The more distantly related genus *Dracaena* (*D. arborea*) also has about 38 somatic chromosomes.

The species of *Agave* studied have 5 pairs of large chromosomes and 25 pairs of small chromosomes. The chromosomes of *A. virginica* and of *A. americana* are shown at different stages of the first meiotic division (figures 4, 5, and 6). Aceto-carmine smears of *Agave consociata* also show the same chromosome number and morphology. According to Mr. S. Horovitz of the University of Buenos Aires, *Agave filifera* also has 5 large and 25 small pairs of chromosomes.

The Agavoideae include according to Engler and Prantl the genera

Agave, Polianthes, Bravoa, Furcraea (*Fourcroya*) and *Beschorneria,* all natives of central America, and the Australian genus *Doryanthes.* According to Heitz (1926) *Furcraea altissima* has 10 large somatic chromosomes and about 40 small ones while *F. Lindeni* has 10 large chromosomes but only 30 small ones. We have found 10 large chromosomes and 50 small ones in aceto-carmine preparations of root tips from *Furcraea Bedinghousii,* but in order to get satisfactory preparations it was necessary to isolate single dividing cells and flatten the metaphase chromosomes by heat and slight pressure. According to Heitz, Müller's work on *Beschorneria* shows chromosome numbers similar to those of *Furcraea.*

As a rule pollen grain size is generally correlated with chromosome number in closely related species. The size and morphology of the pollen grains appear to be very similar in the following species;—*Yucca macrocarpa, Y. Treculeana, Y. baccata, Y. elata, Y. Reverchoni, Y. Thompsoniana, Y. mohavensis, Clistoyucca brevifolia, Hesperaloe parviflora, Hesperoyucca Whipplei, Agave Havardiana* and several unidentified species or varieties of *Yucca* and *Agave.* Each species and variety examined had almost 100 percent of morphologically perfect pollen grains.

DISCUSSION

The confusing variation within species and the intermediate forms of *Yucca* might be interpreted as the result of extensive hybridization between species and varieties. All of the species examined show regular chromosome behavior at meiosis and practically all of the pollen is morphologically perfect. If the questionable forms are hybrids the parental varieties or species must be closely related and possess very similar genoms. In respect to chromosome number and chromosome morphology all of the species studied seem to be very similar. If the chromosomes of different species are compatible with each other, a considerable amount of crossing might be expected and would be limited only by geographic isolation and differences in time of flowering. The cytological analysis alone offers little help in solving the cause of variations within and between species of *Yucca.*

The striking similarity of the chromosomes of *Yucca* and *Agave* and their allied genera indicates a close relationship between these two groups, even though they are referred to different families. The chromosome constitution, 5 pairs of large chromosomes and 25 pairs of small chromosomes, is so unusual that it cannot be due to chance. The two genera are also similar in many taxonomic characters. A brief comparison of the two genera, and of the families to which they

belong, has been prepared by Dr. Ivan M. Johnston of the Arnold Arboretum, and is quoted below.

"*Yucca* belongs to the Liliaceae and *Agave* to the Amaryllidaceae. The Amaryllidaceae, having an inferior rather than a superior ovary, are evidently more specialized in basic floral structure. Taken as a whole the two families differ only in this character of the ovary. There is every evidence that the Amaryllidaceae have been derived from the Liliaceae and there are some very good reasons to suspect that the former are a polyphyletic group with the several points of origin in the Liliaceae.

Yucca and *Agave* are similar in many striking details. Both are coarse fibrous perennials with usually firm, long-enduring, monocarpic leaf-rosettes that are not common in their families. The large panicles are similar in basic structure and pattern.

Yucca has a superior ovary; the filaments are attached at the base of the corolla, bearing small firmly affixed anthers which do not surpass the corolla-lobes.

Agave has an inferior ovary; the stamens are attached in the corolla throat, the linear filaments bear large linear versatile anthers usually protruding beyond the corolla-lobes.

While *Yucca* is more simple than *Agave* in structure of ovary, and presumably belongs to a generally more primative family, its staminal structures and the complex symbiotic relation required in its pollination are distinctly far in advance over conditions found in *Agave*. It is evident, therefore, that *Agave* could scarcely have been evolved from *Yucca* (including *Samuela, Hesperoyucca, Cleistoyucca* and *Hesperaloe*) as now constituted.

Yucca is generally accepted as having relations in *Nolina, Dasylirion* and *Dracaena* of the Liliaceae. *Furcraea,* in the Amaryllidaceae, is a relative of *Agave* although it possesses many important details of habit and floral structure very similar to those found in the relatives of *Yucca*. If *Yucca* is a reasonably close relative of *Agave,* as I believe it is, then it is probable that the affinity is to be traced through the related genera mentioned."

In the Dracaenoideae only the Yucceae have 5 large and 25 small pairs of chromosomes. All of these chromosomes have terminal spindle fiber attachments. In the two species examined (*Nolina* sp. and *Dracaena arborea*) in the Nolineae and Dracaeneae the somatic chromosome number seems to be about 38. These chromosomes do show considerable size differences but not as extreme as in *Yucca,* and the spindle fiber attachment constrictions seem to be median.

In the Agavoideae the two genera studied, *Agave* and *Furcraea,* have

chromosomes very similar to those of *Yucca*. The cytological comparison shows a very close relationship between the Yucceae and certain genera of the Agavoideae, but *Nolina* and *Dracaena* do not seem to be the connecting link between these groups.

According to Engler and Prantl, all genera of the Yucceae, and with the exception of the Australian genus *Doryanthes*, all genera of the Agavoideae, are natives of Central America. These genera are closely related as indicated by both taxonomic and cytological characteristics. It is of interest to note that the African Aloinae, including the genera *Aloe, Gasteria, Apicra* and *Haworthia*, are in some respects similar to the Yucca-Agave group. All of these African genera have the same chromosome number, and the chromosome complex includes both large and small chromosomes. There are 4 pairs of large chromosomes and 3 small ones in each of these genera (Gaiser, 1930). The morphological characters and the similarity in size differentiation of the chromosomes seems to indicate a remote affinity between the Aloinae of the Old World and the Yucca-Agave group of the New World.

SUMMARY

Yucca and *Agave* are similar in many taxonomic characters although one genus is placed in the Liliaceae and the other in the Amaryllidaceae. *Yucca* and the closely allied genera *Hesperoyucca, Hesperaloe,* and *Samuela,* have 5 pairs of large chromosomes and 25 pairs of small chromosomes at the meiotic divisions. Exactly the same chromosomes constitution is found in *Agave* and in at least one species of the closely related genus *Furcraea*. The similarity in taxonomic characters and chromosome constitution indicates that these genera have had a common origin and are closely related.

The variability within and between species of *Yucca* is not correlated with any variation in chromosome number or irregularity in chromosome behavior.

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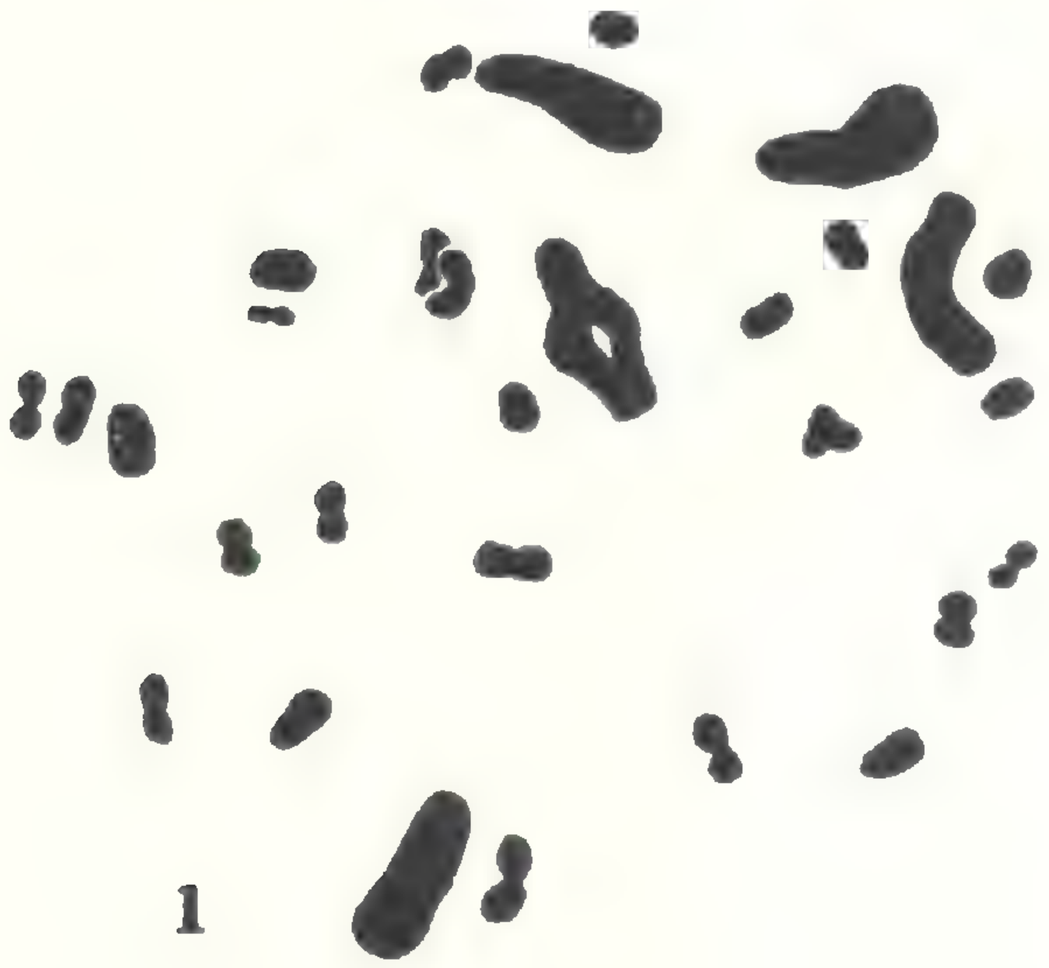

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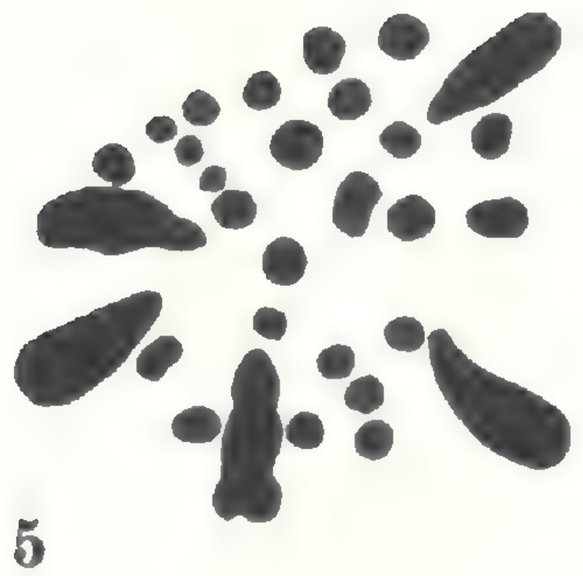

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TAXONOMIC AND CYTOLOGICAL RELATIONSHIPS OF YUCCA AND AGAVE.

DESCRIPTION OF PLATE 55

The drawings were made from permanent smears of pollen mother cells. Magnification about 2500.

1. *Yucca flaccida.* Diakinesis.
2. *Yucca filamentosa.* Metaphase.
3. *Yucca flaccida.* Telophase.
4. *Agave virginica.* Diakinesis.
5. *Agave virginica.* Metaphase.
6. *Agave americana.* Telophase.

CHROMOSOME NUMBERS IN ULMUS AND RELATED GENERA

KARL SAX

With plate 56

The genus Ulmus contains about 18 species and a considerable number of varieties and hybrids (Rehder 1927). The genus is represented in the temperate regions of North America, Asia and Europe. Most species develop their pollen grains and flower early in the spring, but two species, *U. parviflora*, the Chinese Elm, and *U. serotina*, from south central United States, flower in early autumn. The other species have well developed flower buds in the fall but do not bloom until early spring.

Chromosome counts at meiosis were obtained from aceto-carmine smear preparations. Fourteen pairs of chromosomes were found in the following species and varieties,—the American species, *U. racemosa* and *U. fulva;* the European species, *U. laevis, U. glabra, U. procera purpurea* and *U. foliacea;* and the Asiatic Elms, *U. laciniata* and *U. japonica*. *Ulmus hollandica* varieties *Pitteursii* and *superba* also have 14 pairs of chromosomes, although there is occasionally some irregularity in the meiotic divisions of the variety *superba*. The American Elm, *U. americana* is a tetraploid with 28 pairs of chromosomes. There is, as might be expected, some indication of secondary chromosome pairing in the species. The variety *pendula* is also a tetraploid. It is probable that *U. americana* is an autotetraploid and that only bivalent chromosomes are formed because of the limited chiasma frequency.

The numerous varieties of *U. hollandica* are presumably segregates from hybrids between *U. glabra* and *U. foliacea*. The two varieties studied show only slight irregularities in meiotic divisions and pollen fertility is almost complete. It seems probable that a number of species hybrids could be made in this genus.

During the spring of 1932 crosses were made between *U. americana* and *U. laevis*. A considerable number of seeds were obtained, but owing to technical difficulties in pollination it is probable that many of the seeds were not of hybrid origin. Since *U. americana* is a tetraploid and *U. laevis* a diploid, the hybrid seedlings should be triploids. Chromosome counts from these seedlings have not been completed, but the seedlings examined seem to be tetraploids.

In general, the species of Ulmus constitute a rather closely related

group as indicated by chromosome number and morphology, the ocurrence of fertile species hybrids, and the similarity of certain Old and New World species.

The related genus *Zelkova*, also has 14 pairs of chromosomes. The reduction divisions in *Z. serrata* seem to be regular.

The monotypic genus *Hemiptelea* is apparently a polyploid with a large number of chromosomes which are irregularly distributed at the meiotic divisions. About 70-80 per cent of the pollen of *H. Davidii* is morphologically imperfect.

Celtis occidentalis also has very irregular meiotic divisions. The diploid chromosome number is 28, but at the first meiotic division there is little chromosome pairing and the univalent chromosomes are irregularly distributed to the daughter nuclei. In some cases 28 univalents were observed at the first meiotic metaphase stage and in most cases only a few bivalents were found (Fig. 11). The chromosome behavior was the same in two different trees and in both cases at least 80 per cent of the pollen was sterile. A considerable number of fruits were produced on both trees.

The irregular chromosome behavior and high pollen sterility in a good species is difficult to understand. If this species contains two different basic sets of chromosomes with only a few homologous chromosomes or parts of chromosomes it could breed true only by some form of apomixis. Such true breeding hybrids are found in nature. *Malus theifera* for example is a triploid which produces no functional pollen, but it reproduces the type and has a natural distribution.

It is also possible that the prevalence of galls on *Celtis* may influence the meiotic divisions. Kostoff and Kendall (1929, 1930) have found that gall mites and other parasites may cause chromosome irregularity in certain solanaceous plants. Although no parasites were observed on the Celtis flowers collected it is perhaps possible that the residual effect of constant earlier infections may have had an influence on the meiotic divisions. If megasporogenesis is less easily disturbed a considerable number of fruits might be produced even though relatively few of the pollen grains are functional. Additional work on *C. occidentalis* and other species should be done to determine the cause of pollen sterility in this genus.

The basic chromosome number is 14 for all of the genera of the Ulmaceae investigated. Among the allied families the basic chromosome number is 16 for the Juglandaceae (Woodworth, 1930), 8 and 14 for Betulaceae, (Woodworth, 1931), 12 for the Fagaceae (Sax, 1930), and 14 for several genera in the Moraceae (Gaiser, 1930). From the standpoint of chromosome numbers the Ulmaceae might seem to be

allied with the *Alnus, Betula, Corylus* group in the Betulaceae and *Morus* in the Moraceae, but the great diversity in morphological and anatomical characters would not seem to indicate any close phylogenetic relationships among these families.

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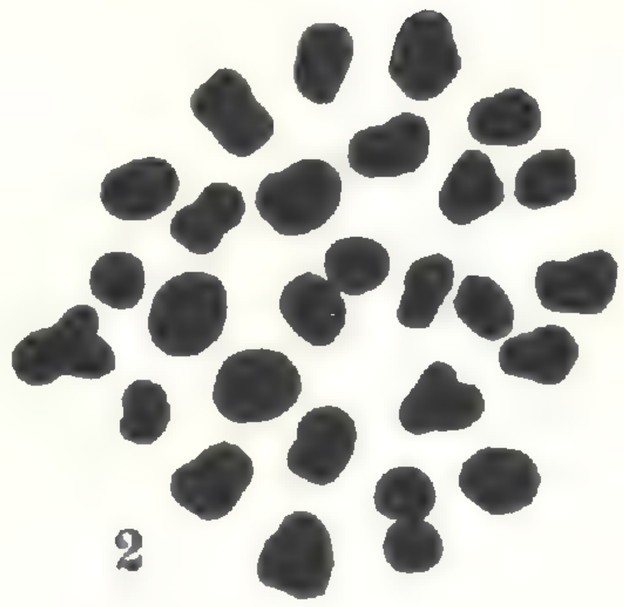
DESCRIPTION OF PLATE 56

Drawings from aceto-carmine preparations of pollen mother cells. × 2000. First meiotic metaphase shown in all figures except number 10 which is from a second metaphase plate.

Figure 1. *Ulmus laevis.*
Figure 2. *Ulmus americana pendula.*
Figure 3. *Ulmus racemosa.*
Figure 4. *Ulmus fulva.*
Figure 5. *Ulmus laciniata.*
Figure 6. *Ulmus procera purpurea.*
Figure 7. *Ulmus japonica.*
Figure 8. *Ulmus hollandica Pitteursii.*
Figure 9. *Ulmus foliacea.*
Figure 10. *Zelkova serrata.*
Figure 12. *Celtis occidentalis.*


1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MYCORRHIZAL AND OTHER FEATURES OF THE ROOT SYSTEMS OF PINUS

A. B. Hatch and K. D. Doak

With plates 57-60

The distinctive anatomical and morphological features of mycorrhizal and non-mycorrhizal root systems, as illustrated by those of *Pinus*, are the subject matter of this paper. The discussion, although limited to *Pinus*, considers some features that are common to all ectotrophic mycorrhizal organs of woody plants.

The term ectotrophic mycorrhiza refers to a distinct morphological organ consisting of a "short root" and a fungus that are constantly arranged in an orderly manner with reference to each other. It is a well-defined organ and need not be confused even with its nearest relative the pseudomycorrhiza. Frank (1885) coined the term "mycorhiza"—a compound of Greek terms meaning "fungus root." He described the structure as neither root nor fungus, but rather—analogous to the thallus of the lichen—an association of two differing components to form one morphological organ.[1]

The use of the term mycorrhiza in reference to ectotrophic structures (under which we also include the ectendotrophic forms for con-

[1]In his first paper on mycorrhiza with reference to the absorbing roots of Oak, Beech, etc., Frank stated that they consist of two kinds of elements—(1) "aus einem Kern, welcher die eigentliche Baumwurzel repräsentiert, und" (2) "aus einer mit jenem organisch verwachsenen Rinde, welche aus Pilzhyphen zusammengesetzt ist." "Der ganze Körper ist also weder Baumwurzel noch Pilz allein, sondern ähnlich wie der Thallus der Flechten, eine Vereinigung zweier verschiedener Wesen zu einem einheitlichen morphologischen Organ, welches vielleicht passend als Pilzwurzel, Mycorhiza bezeichnet werden kann." (Frank, 1885, p. 129.)

The frequent subsequent use of the term mycorrhiza (or mycorhiza) to describe a condition rather than an organ is the result of the absence of such a definite organ in Orchidaceae and Ericaceae, where endophytic infection by one mycelium may occur in roots, stem, leaves and seeds. The usages of the one term to indicate either a condition or an organ are not compatible; consequently it would seem advisable to extend our terminology. One of the difficulties of the dual meaning is the use of the singular noun in the plural sense. Thus the singular "mycorrhiza" is frequently employed in referring to a number of short roots that collectively have been converted into "mycorrhizae." In view of Frank's original definition this is obviously incorrect.

The word mycorrhiza is compounded from the Greek μύκη, a rare variant of μύκης "fungus," and of ῥίζα "root," with ῥῥ (rrh) medially (as usual) and αί in the plural (since ῥίζα is a feminine noun of the first declension), which in botanical usage is latinized, becoming *"ae."* Most authors have adopted the plural endings of their respective languages in their writings on ectotrophic mycorrhizae, but in English this is awkward phonetically. Kelley (1931) therefore adopted the latinized Greek plural ending, while retaining the single "r" of Frank. The spelling employed in this paper is the one considered preferable by authorities on the Greek language.

venience) is restricted by most European investigators to such an organ formed from short roots. Elsewhere, the term is frequently used to indicate indiscriminate fungal attacks on "long" as well as on "short roots." McArdle (1932) for example has recently published photographs (Figs. 1 to 6 and plate 2) of root systems of Pine and Spruce grown in open culture synthesis experiments to support his belief that certain fungi investigated form mycorrhizae with the roots of those trees. In figure 5 (ibid.) a dead radicle is illustrated from which three replacement radicles have emerged and grown geotropically. These are labeled "Mycorrhizae on Norway spruce, formed in synthesis with *Lycoperdon gemmatum*." It is evident from this photograph and from the others mentioned above that long roots are considered to be mycorrhizal organs. Neither these nor any of the visible short roots, however, exhibit the external characters possessed by a typical mycorrhiza. Since conversion of root tips into mycorrhizal structures is probably dependent on slow elongation rates it is improbable that long roots (rapidly growing roots) can be converted into "fungus roots" while the short roots (slow growing roots) of the system remain uninfected. Again, among 21 photographs and drawings of roots of culture plants reported to be mycorrhizal by Masui (1927, figs. 63-84) one drawing only (fig. 83) is included that illustrates a structure characteristically mycorrhizal (ectotrophic). Illustrations of non-mycorrhizal intracellular infection, of cells evidently taken from long roots, are common (figs. 75 and 76).

Further confusion in mycorrhizal studies has resulted from failure to qualify the use of the terms "large root" and "rootlet." The latter evidently has been used to cover all elongating roots (McArdle, 1932). In connection with the former, Masui (1927, fig. 90) and others have used microchemical methods for studying the physiological functions of mycorrhizal roots and in so doing have apparently compared mycorrhizal "short roots" with non-mycorrhizal "large" or "long roots." The impropriety of comparing such roots microchemically may account for the diversity of opinion among these investigators concerning the physiology of mycorrhizal associations.[1]

The profuse surface growth of non-mycorrhizal and probably parasitic fungi on roots has frequently caused such roots to be mistaken for true mycorrhizal structures. Thus abundant and apparently parasitic

[1]McArdle (1932, p. 292) states that all investigators who have used this technique agree that "mycorrhizal fungi are parasitic upon the associated higher plants, and that there is no evidence of a symbiotic relationship." However, Rexhausen (1920) and Aali (1923) came to views exactly opposite, namely that mycorrhizal fungi are beneficial to the associated higher plant; Weevers (1916) is uncertain of the relationship, and Weyland (1912) and Masui (1927) conclude that parasitism exists.

attacks (with sporophore production) of certain Gasteromycetes on mycorrhizal roots, roots already mycorrhizal through association with Hymenomycetes, have recently been observed in several instances by the authors (further communication by Doak). Recognition of the nature of such secondary attacks is often extremely difficult, a fact which undoubtedly accounts for much confusion in the literature.

Attempts to learn of the abundance of the mycorrhizal roots of trees by reviewing the world's literature of these structures are singularly futile. With few notable exceptions investigators have either neglected to define their use of the term "mycorrhiza," failed to apply it exclusively (and quantitatively) to those roots known as short roots (absorbing roots, "Saugwürzelchen oder Ernährungswurzeln") or omitted a tally and description of the roots they have not listed as mycorrhizal.

In America, McDougal (1928) has reported repeatedly upon the existence of mycorrhizal structures on forest tree roots. One gathers from his papers that although they are widely distributed they are relatively nonabundant and in fact are somewhat infrequent if present at all on some species. For a number of years the authors have devoted their attention, almost exclusively, to the study of the mycorrhizal roots of forest trees both in America and Scandinavia. During this period they have rarely observed short roots (on forest trees over one year in age, growing in good forest soil with pH less than 5.5) that were not mycorrhizal. This is in line with the observations of numerous investigators, including Frank (1885) in Germany who recorded the invariable conversion of short roots into mycorrhizal roots, and also including Melin (1917, 1923, 1925 and 1927) in Sweden. Kelley (1932) reports similar phenomena for practically all woody plants he has examined in the eastern and middle western United States during the past four years.

The apparent lack of agreement among workers in this field arises in part from failure to distinguish the various root forms. The diagnostic characteristics of the several types of roots occurring on Pine are discussed below.

CLASSIFICATION OF PINE ROOT SYSTEMS

Root systems of conifers exhibit several kinds of roots and are therefore called "heterorhizic" by Noelle (1910). Two broad types are distinguished: (1) long roots ("Bereicherungswurzeln") and (2) short roots or absorbing roots ("Kurzwurzeln, Saugwürzelchen" or "Ernährungswurzeln"). Anatomically these differ in *Pinus* as follows:

1. Long roots are furnished with root caps, while short roots lack

this structure, having a few layers only of cells beyond the plerome apex. (Plate 57, A and B.)

2. Long roots are diarch or polyarch, while short roots are monarch. (Plate 57, D.)

3. The diameter quotient, that is, ratio of stele diameter to total diameter, of short roots is significantly lower than that of all other types of roots (Aldrich-Blake, 1930, p. 24).

4. Long roots exhibit secondary growth, while this condition has never been observed in short roots (Noelle, 1910, reports a questionable observation to the contrary).

5. Root hairs arise from the second or third layer of cortical cells in long roots (Plate 59, D; Noelle, 1910; Aldrich-Blake, 1930) and from the epidermal cells in short roots when the latter possess these structures (Plate 57, B and D).

6. Long roots branch racemosely, while short roots branch dichotomously only. (Text fig. 1 and plate 58, C.)

In addition to anatomical differences these roots have vastly different elongation rates. Finally, barring accidents, long roots, in nature, are permanent structures, while short roots are ephemeral.

It should be mentioned that the monarch structure in uninfected short roots is demonstrated for the first time in this paper (Plate 57, D). Previous workers have apparently considered that the monarch structure resulted from infection by mycorrhizal fungi. Noelle (1910, p. 254), for example, writes—"Die unverpilzten Ernährungswurzeln besitzen dagegen einen wohl ausgebildeten diarchen Protoxylemstrang, . . ." The materials Noelle studied were all from soils. It is probable, therefore, that most of the short roots were converted into mycorrhizal structures, and that Noelle's "unverpilzten Ernährungswurzeln" were in actuality arrested diarch lateral root initials. The latter are described in detail below in the section headed "Types of long roots."

The gross characteristics of long and short roots are illustrated in Text figure 1. The root system of a seedling grown in a mixture of sand and forest humus (the infektionsjord of Hesselman, 1927, p. 361; also Gast, unpublished) is shown. It has 24 long roots (totaling 65 cm. in length) in addition to a 25 cm. radicle or tap root. Including arrested laterals it had 435 short roots (quite a few of which were broken off in transit to America before the photograph was taken). Arrested laterals are to be noted along the radicle and on one uppermost side branch. A slow-growing lateral that was not attacked—as evidenced by absence of swelling and of dichotomy in the basal portion—by a mycorrhizal fungus until it had reached a length of about 1 cm. is marked by the digit "4." The short roots are either mycorrhizal or

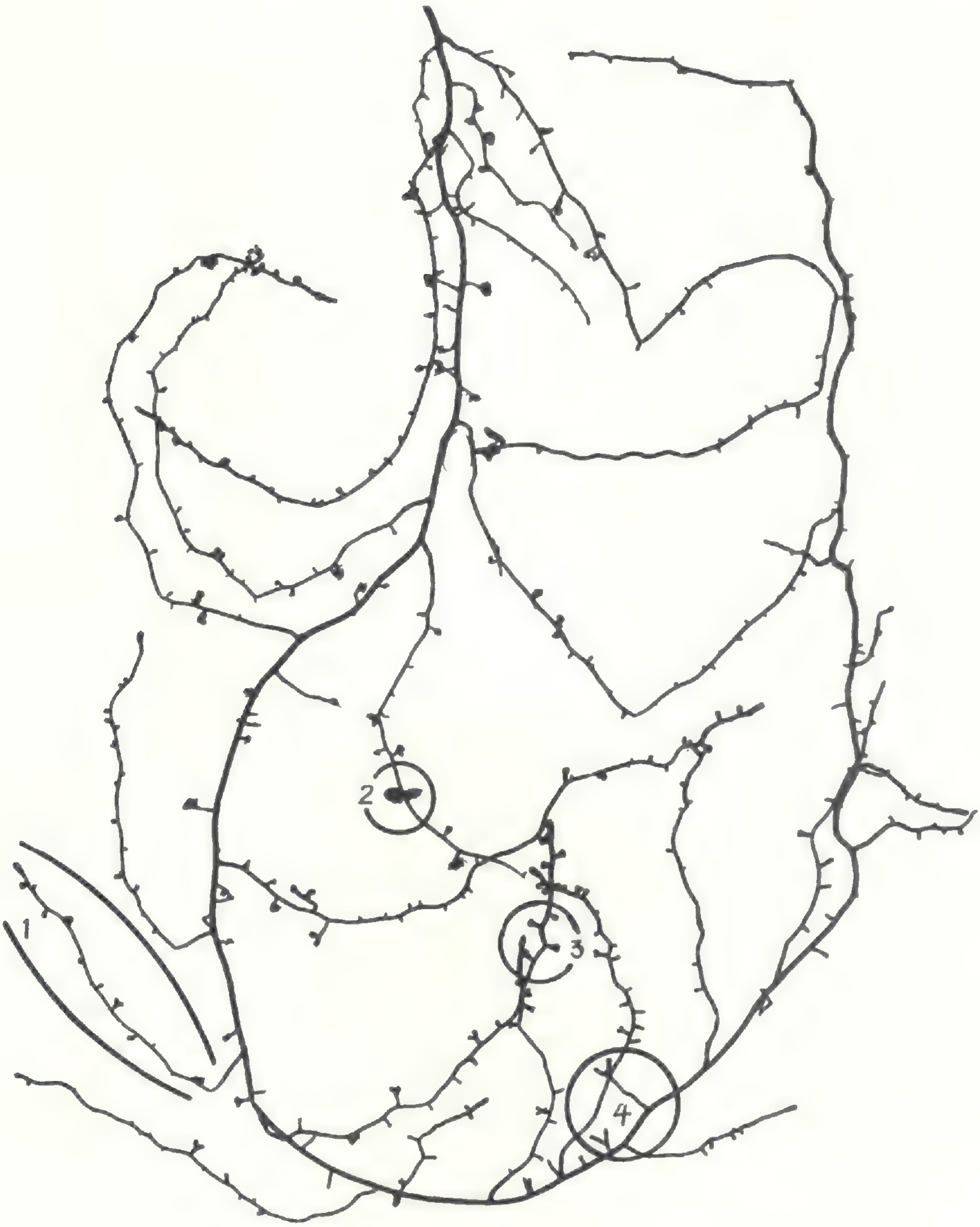

FIGURE 1. ROOT SYSTEM OF PINUS.

Entire root system of a three months old seedling of *Pinus sylvestris* L. grown in sand-mixed humus (infeksionsjord Jönåker, Sweden; unpublished material of P. R. Gast's, Harvard Forest); × 1.58. (1) Mother root, the tip of which is mycorrhizal; (2) short roots attacked by *M. R. atrovirens* type of fungus; (3) dichotomously branched short roots; (4) arrested lateral root initials.

pseudomycorrhizal. Mycelium of the *Mycelium radicis atrovirens* type (Melin, 1923, p. 223) is visible on many of the short roots. The longest true short root on the system does not exceed 3 mm. in length.

The comparative abundance of long and short roots on experimental seedlings is given in Table 1. These seedlings had an average of 19 long roots and 474 short roots per seedling. Thus 96 per cent of the growing root tips are short roots. This is of considerable significance when it is considered that ordinarily these are all mycorrhizal, while the remaining 4 per cent—long root tips—on the other hand rarely acquire this structure.

TYPES OF LONG ROOTS

The term "long root" includes (a) the radicle or tap root (also a replacement radicle should the original radicle be injured), (b) diarch laterals called "mother roots" and (c) polyarch continuations of mother roots termed "pioneers." Of these the radicle is the most strongly developed root in young seedlings. It is polyarch, has a large diameter quotient and a large and well-developed root cap. Lateral roots emerge from the radicle. These either abort (see below) or become mother roots. The latter are diarch and are characterized by a moderate elongation rate and by the production of numerous short roots and secondary laterals. At variable distances from their points of origin mother roots are converted into pioneer roots by augmentation of the number of protoxylem strands. (In Scots Pine, however, all long roots remain diarch, Noelle, 1910). Pioneer roots are the most poorly-branched members of pine root systems, largely because of abortive secondary root initials (Aldrich-Blake, 1930). Pioneer roots elongate rapidly, have an intermediate diameter quotient, and a well-developed root cap. Collectively these roots markedly increase the spread of root systems. They likewise bear lateral and secondary roots in abundance (particularly true of mother roots). To what extent the growing tips of long roots, where the conduction systems are undifferentiated, function as absorbing organs is known by speculation only. The early development of secondary endodermis a short distance back of the tips presumedly soon prevents absorption. Most workers have assumed that absorption is accomplished mainly by short roots. An experimental background for the assumption is lacking. Although a long root is capable of being converted into a mycorrhiza this condition is comparatively infrequent (Melin, 1927).

A knowledge of the behavior of diarch lateral root initials is essential to a proper understanding of the mycorrhizal habit. Three destinies are possible as follows: (1) they may abort before or during emergence or before they have reached an appreciable length; (2)

TABLE 1. ROOT MEASUREMENTS OF 3 MONTHS OLD PINUS SYLVESTRIS L. SEEDLINGS GROWN IN SAND MIXED HUMUS

Seedling number	Number of long roots Total	Number of laterals over 1.5 cm. long	Length of radicle cm.	Total length of laterals cm.	Length of longest lateral cm.	Total length of all long roots cm.	Number of short roots	Number of short roots per 10 cm. of long roots
1	21	16	26	54	6	80	596	77
2	17	12	27	51	5	78	489	74
3	21	12	25	53	10	78	504	70
4	27	18	25	90	11	115	535	55
5	19	10	23	59	7	82	540	72
6	21	18	18	97	11	115	700	70
(Average 6)	21.0	14.3	24.0	67.3	8.3	91.3	560.3	69.7
1	16	12	17	63	11	80	304	47
2	13	9	17	41	7	58	400	76
3	18	13	20	50	6	70	399	57
4	16	8	21	30	4	51	272	78
(Average 4)	15.8	10.5	18.8	46.0	7.0	64.8	343.8	64.5
Average 10	18.9	12.8	21.9	58.8	7.8	80.7	473.7	67.6

Upper six seedlings grown in inoculation earth (infecktionsjord, Hesselman, 1927) in 50% solar radiation minus short ultra-violet (λ 0.31 to 0.30 μ) and infra-red; lower four in good raw humus (ibid.) in 50% solar radiation minus ultra-violet; all were grown at the Royal Institute of Experimental Forestry, Stockholm, Sweden.

they may be attacked by microörganisms with resultant conversion into mycorrhizal or pseudomycorrhizal structures; (3) they may, by continued growth, become mother roots. The latter in turn give rise to initials having the same possible destinies and to monarch short roots.

The distribution of lateral root initials along the radicle in Corsican Pine, their growth and their anatomical features have been studied by Aldrich-Blake (1930). He found that seedlings grown in a sandy soil produced initials opposite any of the four protoxylem points at distances of 1.6 to 2.7 mm. apart. One-half of these became abortive before they grew one millimeter in length and only one-sixth grew beyond the very moderate length of 10 mm. The termination of growth in many initials at an early stage was associated with mycorrhizal formation. The question of whether they were predestined to become mother roots or mycorrhizal roots was therefore examined. Internal as well as external factors were judged to be operative as evidenced by the finding of an abortive initial that had ceased growth while still within the intact endodermis of the parent radicle (l. c., p. 22). It was further determined that the initial size of the roots which became mycorrhizal was distinctly smaller than that of those which continued growth and became mother roots. Aldrich-Blake writes, for example—"The mean protoxylem diameter of the mother roots was 117 ± 6 μ, while that of the diarch basal portion of the dichotomized roots was 73 ± 3 μ; and the probability of significance of the difference between these two measurements was over 100 to 1" (l. c., p. 23). He concluded that the two types of roots were distinct from the very beginning in respect to size and that conversion to mycorrhizal organs was "the response of feeble roots to strong fungal infection" (l. c., p. 24). Melin (1925) also presents convincing evidence that many laterals are "feeble" from the very beginning. It is apparent from photographs (Melin, l. c., pp. 60-62, figs. 23-27) of *Pinus sylvestris* that even in the complete absence of microörganisms and in a uniform substratum the common differentiation of laterals into slow-growing roots and mother roots still occurs. After three years many of the slow-growing laterals on pure culture seedlings had not reached the length of one centimeter (l. c., 1925, p. 61).

During examinations of approximately 2500 seedlings of *Pinus sylvestris* L. and *P. Strobus* L. grown in pot cultures (containing mixtures of forest humus or soil and sand) one of the authors has observed that only in the autumn, after elongation of the rapidly growing long roots has nearly ceased, do the tips of the latter frequently acquire the mycorrhizal structure (Plate 59, A). This leads us to believe, especially in view of the slow elongation rate of short roots which normally are always converted into mycorrhizal organs, that rapid elongation is

the primary reason long roots ordinarily escape attack by mycorrhizal fungi. This hypothesis assumes (1) that mycorrhizal fungi can only successfully attack a root in the primary condition, and (2) that in rapidly growing long roots the secondary condition is acquired before mycorrhizal fungi are able to penetrate sufficiently to initiate the characteristic response. Rayner (1927, p. 99) has in part explained a somewhat similar phenomenon, namely, the absence of the endophyte from rapidly growing roots of *Calluna vulgaris* (L.) Salisb. in the early spring, by assuming differential growth rates for fungus and root at low spring temperatures. The same phenomenon is probably operative in tree seedlings, except that root growth rather than fungal growth is assumed to be retarded by low temperatures or other factors. In this connection a seedling of *Pinus resinosa* Ait. grown under very adverse conditions in pure culture has been observed to have all of its roots (long as well as short) converted into mycorrhizal roots (later communication by Hatch). We conclude, therefore, that every root tip in the primary condition is theoretically capable of conversion into a mycorrhiza. That this normally does not occur in nature we attribute to differences in rapidity and character of growth of long and short roots.

Lateral root initials that become mycorrhizal (Plate 59, B) acquire the monarch structure in the dichotomized branches above their diarch basal portion, and they are ephemeral structures. They undoubtedly function in the same manner as true mycorrhizal short roots. For the purpose of our present discussion, therefore, we may include them in the category of short roots.

TYPES OF SHORT ROOTS

Three distinct kinds of short roots may be distinguished: (1) the uninfected short root, (2) the infected short root in which the mycorrhizal structure is absent (pseudomycorrhiza), (3) the infected short root which has acquired the mycorrhizal structure (mycorrhiza).

The first of these, or uninfected short roots, are distinguished by the following features (Plate 57, B):

1. Formation of root hairs from epidermal cells (when grown in a suitable nutrient solution and in a substratum that is adequately aërated).
2. Continuous although slow elongation.
3. Absence of hypertrophy of cortical cells.
4. Complete absence of fungus infection.
5. Frequent but not profuse dichotomy[1] (Melin, 1925, figs. 23, 24).

[1]It should be noted that dichotomous branching of the short roots is an exclusive habit, so far as is known, of the genus *Pinus* among conifers. The term

This type of short root is exceedingly rare. It appears consistently and probably exclusively in pure cultures and in open, mineral solution sand cultures that are free from organic materials. Root hair production is dependent on the condition of the substratum relative to aëration and chemical composition (Melin, 1925, p. 61). It is notable that root hairs on short roots are elongations of epidermal cells (Plate 57, B and D). Its diagnostic value, considering that all root hairs on long roots of Pine originate from the second or third layer of cortical cells (Aldrich-Blake, 1930, p. 16; Noelle, 1910; and Plate 59, D of the present paper), has not been emphasized. In pure culture short roots exhibit renewed yearly growth in length and have been reported to reach a maximum length of 20 mm. in two years (Melin, 1925, p. 60). The absence of a root cap on mycorrhizal roots is considered to be a natural consequence of attack by mycorrhizal fungi by Laing (1932). McArdle (1932, p. 312) on the other hand attaches physiological significance to the presence of this structure in mycorrhizal roots. That it actually is not present on either uninfected or mycorrhizal short roots in *Pinus* is demonstrated in Plate 57, A, B and C.

The second type of short root exhibits infection, but of a distinctly non-mycorrhizal type. The name pseudomycorrhiza was given to this structure by Melin (1917). Melin applied it to short roots possessing the following features, which likewise are characteristic of American material:

1. Intracellular infection by soil inhabiting fungi that are usually of minute proportions.
2. Absence of root hairs.
3. Absence of hypertrophy in cortical cells of the roots.
4. Complete absence of intercellular net (Hartig's net).
5. Early termination of growth in length (two to three millimeters is usually the maximum length).
6. Occasional dichotomous branching.

The general habit of pseudomycorrhizal roots of *Pinus sylvestris* is illustrated in Plate 58, A. A longitudinal section of one of these is reproduced in Plate 57, A. A common type of infection of the cortical cells is shown in Plate 60, B, C and D, and by Melin (1917, fig. 39). Möller (1902) first called such a structure an "entotrof" mycorrhiza because it was characterized by endophytic infection only. Melin (1917, p. 360) pointed out that it exhibits no similarity to the true endotrophic infection occurring in the *Ericaceae* and *Orchidaceae*.

"Gabelmykorrhiza" was coined by Melin (1923) in his studies on *Pinus* to cover mycorrhizal roots characterized by this type of branching. In this connection McArdle (1932, p. 295) has referred to the coralloid mycorrhiza of Spruce as the "Gabelmykorrhiza" of Melin; but the mycorrhizal short roots of Spruce branch racemosely and should not be confused with the dichotomous type.

Neither may it be confused with an ectotrophic mycorrhiza since the fine endophytic hyphae wander from cell to cell without regard to structure, even occurring in the endodermis and parenchyma cells of the central cylinder (l. c., p. 359). Hyphae never occur in masses in the cells (ibid.). Several species of fungi may be present at once either in abundance or in extreme scarcity. In exterior appearance a pseudomycorrhiza differs from an uninfected short root chiefly in its shorter length and darker color, when the latter does not possess root hairs.

In contrast to the foregoing root types, attack by a mycorrhiza-producing fungus normally results in short roots that possess the following well-known features:

1. Intercellular net between cortical cells (Hartig's net). Plate 59, E; Plate 60, A.

2. Intracellular infection of the cortical cells (which may or may not be discernible; Melin, 1925).

3. A fungal mantle composed of a few to many layers of tightly packed and interlaced hyphae covering the region of cell hypertrophy (Plate 60, A). The mantle has been reported to be almost completely lacking on mycorrhizal short roots of certain experimental seedlings (Melin, 1925).

4. Hypertrophy of the cells of the cortical region.

5. Characteristic profuse dichotomous branching with age (Plate 59, C).

6. Continued elongation of the dichotomous branches under favorable conditions of soil and weather during one growing season.

The external appearance and internal structure of a typical mycorrhiza is illustrated in Plate 57, C; Plate 58, C; Plate 60, A. The cells of the cortical region of an uninfected short root of *Pinus Strobus* grown in pure culture (Plate 57, C) are on the average 29 μ in radial and 61 μ in longitudinal directions. Dimensions of similar cells of a mycorrhizal short root (Plate 57, B) [also pure culture *P. Strobus* and *Lactarius deliciosus* (L.) Fr.] are 43 μ and 40 μ respectively in radial and longitudinal section (average of 15 cells in both cases). Based on the uninfected short root, therefore, the increase in radial size (hypertrophy) of individual cells is 67 per cent. The diameter of the uninfected short root is 300 μ, while the average diameter of the mycorrhiza in this case is 485 μ, an increase of about 62 per cent. Comparative studies of pure culture seedlings indicate that mycorrhizal formation in short roots of Pine is not accompanied by either hyperplasia or hypoplasia. It is evident, therefore, that Laing's statements (1932, pp. 10, 11) that the root cap disappears during the develop-

ment of a pine mycorrhiza and that the number of cortical cells is less as a result of mycorrhizal formation arose from failure to distinguish between long and short roots. Attack by mycorrhizal fungi, if early in the season, almost invariably results in dichotomous branching of the short root. Judging from the exceedingly slow growth of uninfected short roots (see above), including slow growing laterals, the authors see little basis for the numerous references in the literature to the cessation of elongation of roots following attack by mycorrhizal fungi. Laing (1932), in concluding that mycorrhizal fungi exert a retarding influence on growth in length of "rootlets," evidently failed to make comparisons with uninfected short roots or to include data supporting this view. In this connection McArdle (1932) reports that he observed the formation of what he believed to be "mycorrhizae" through glass plates in sand cultures and that elongation did not proceed after attack by mycorrhizal fungi. McArdle's photographs (ibid., figs. 1-6) as well as his measurements demonstrate conclusively that he was dealing entirely with long root tips. That mycorrhizal short roots do grow after attack by the causative fungus is adequately evidenced by profuse dichotomy which probably never occurs in the absence of true mycorrhizal infection. Likewise the present authors have observed continued growth and branching of typical mycorrhizal short roots, individually, in their own pure cultures on several species of Pines in association with several species of Hymenomycetes. The profuse branching of a mycorrhizal short root of *P. Strobus* is illustrated in Plate 59, C. This short root has approximately 50 individual growing tips; another growing beside it had 84; in fact, all of the short roots on the long root from which these were taken were much branched. We can find no reason to assume that the total length (from the base of the mycorrhiza to the tip) of any of the numerous tips is less than the total length of the short root would have been had it remained non-mycorrhizal. Instead, multiple tip growth represents a greatly accelerated development which increases the absorbing surface many times. In this connection, it is to be noted that by means of hypertrophy of the cortical cells and increase in surface area by means of absorbing fungal hyphae the surface area of a single-tipped mycorrhizal short root seemingly already exceeds that of an uninfected one. A comparative study of the actual absorbing power of mycorrhizal and non-mycorrhizal short roots should yield interesting results.

We have shown that anatomically and morphologically a short root, the organ ordinarily involved in an ectotrophic mycorrhiza, differs markedly from a long root. It seems logical to assume that these root

types differ as fundamentally in their physiological functions. In investigations designed to elucidate mycorrhizal nutrition, therefore, it is essential that these differences be recognized and that the types and subgroups of roots be adequately separated. Similarly in ecological studies, when the abundance of mycorrhizal roots is given in percentage or other figures, these data should apply to short roots only; and these should be separated into the three subgroups—mycorrhizal, pseudomycorrhizal and uninfected short roots respectively.

The authors jointly wish to recognize their indebtedness to the Allegheny Forest Experiment Station maintained at Philadelphia in coöperation with the University of Pennsylvania. The senior author also gratefully acknowledges his indebtedness for advice and facilities given by Professor Elias Melin, Mycological Laboratory, Royal Academy of Forestry, Stockholm, Sweden; by Professor H. Hesselman, Royal Institute of Experimental Forestry, Stockholm; Professors R. T. Fisher and P. R. Gast, Harvard Forest; and Professors Oakes Ames and J. H. Faull, Arnold Arboretum, Harvard University. The senior author is further indebted to the American Scandinavian Foundation for stipends to study in Sweden, as Fellow of the Foundation; and to the Arnold Arboretum for support that enabled him to continue the work at Harvard University, under the auspices of the Arboretum and the Department of Botany, as a graduate student.

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Allegheny Forest Experiment Station, Bureau of Plant Industry, U. S. Department of Agriculture.

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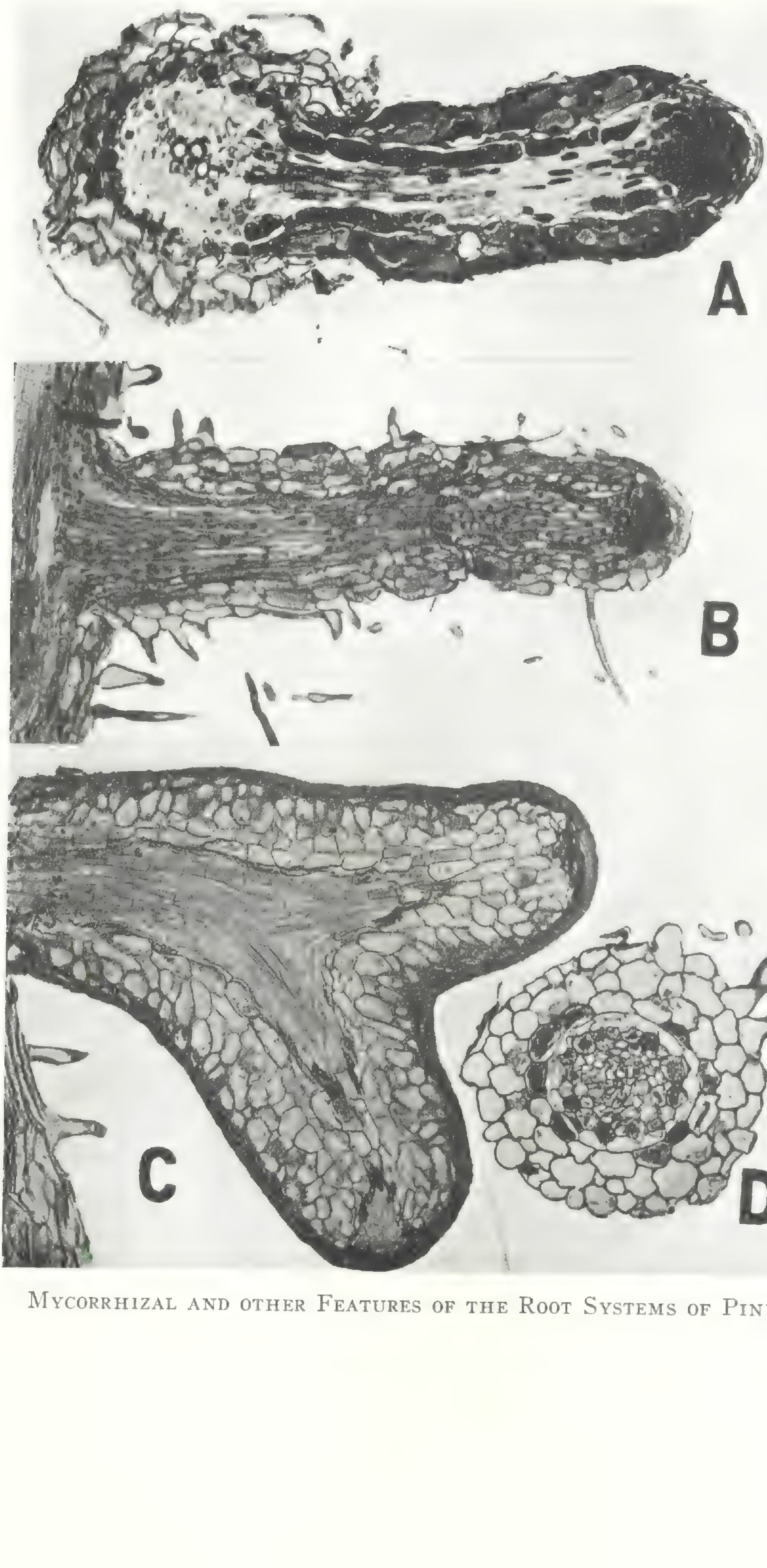
DESCRIPTION OF PLATES

Plate 57. A. Longitudinal section of a pseudomycorrhiza of *Pinus sylvestris*; × 119. (Unpublished results of a growth experiment with Swedish soils of varied carbon nitrogen ratios conducted by Professor E. Melin and the senior author.) See also Plate 58, A.

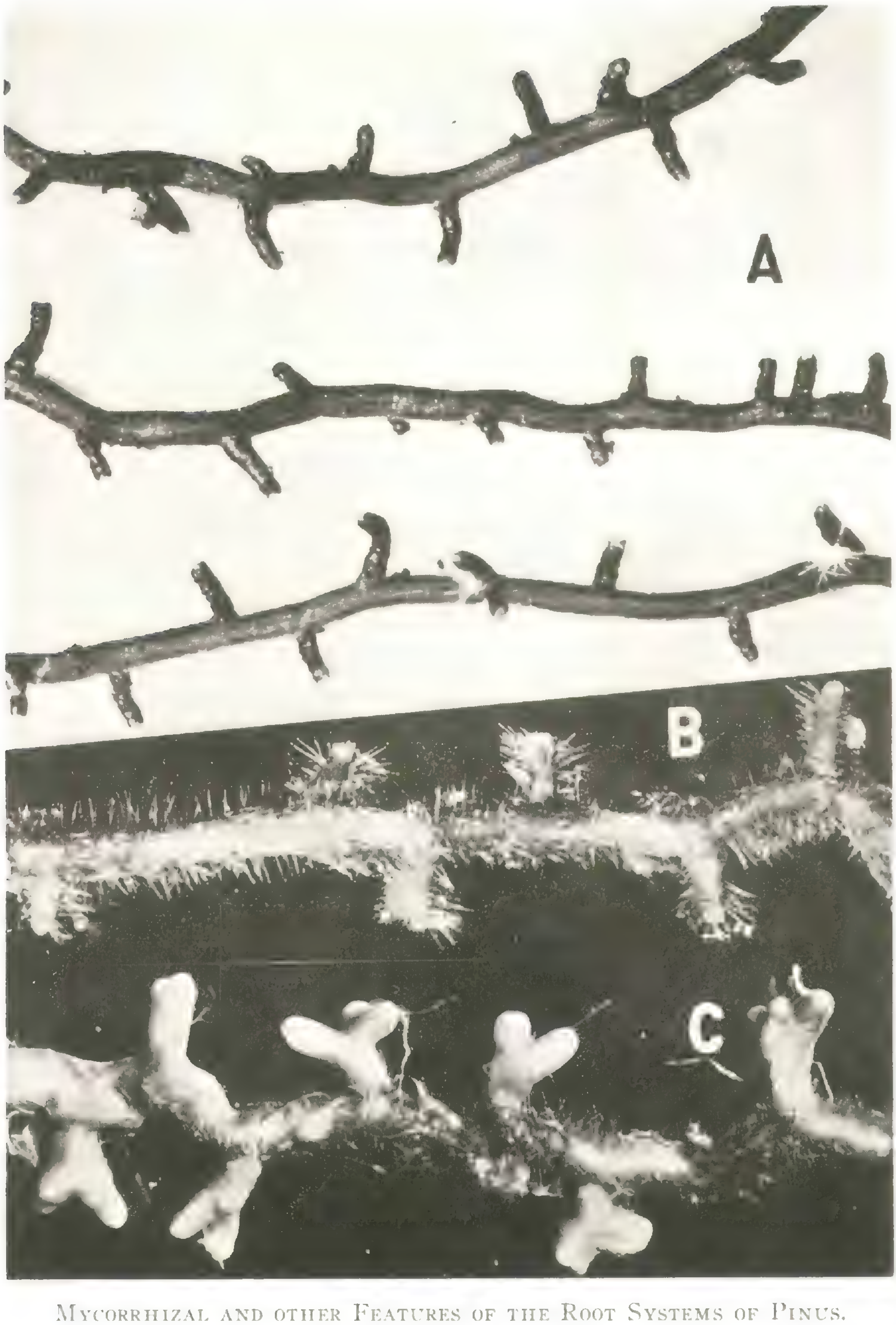
B. Longitudinal (not medial) section of *P. Strobus* short root grown in pure culture, showing absence of hypertrophy and a root hair arising from an epidermal cell; × 69.

C. Longitudinal (not medial) section of dichotomized mycorrhizal short root of *P. Strobus* grown in pure culture with *Lactarius deliciosus* showing mantle, intercellular net and cell hypertrophy; × 67. (Ditto; Plate 58, C.)

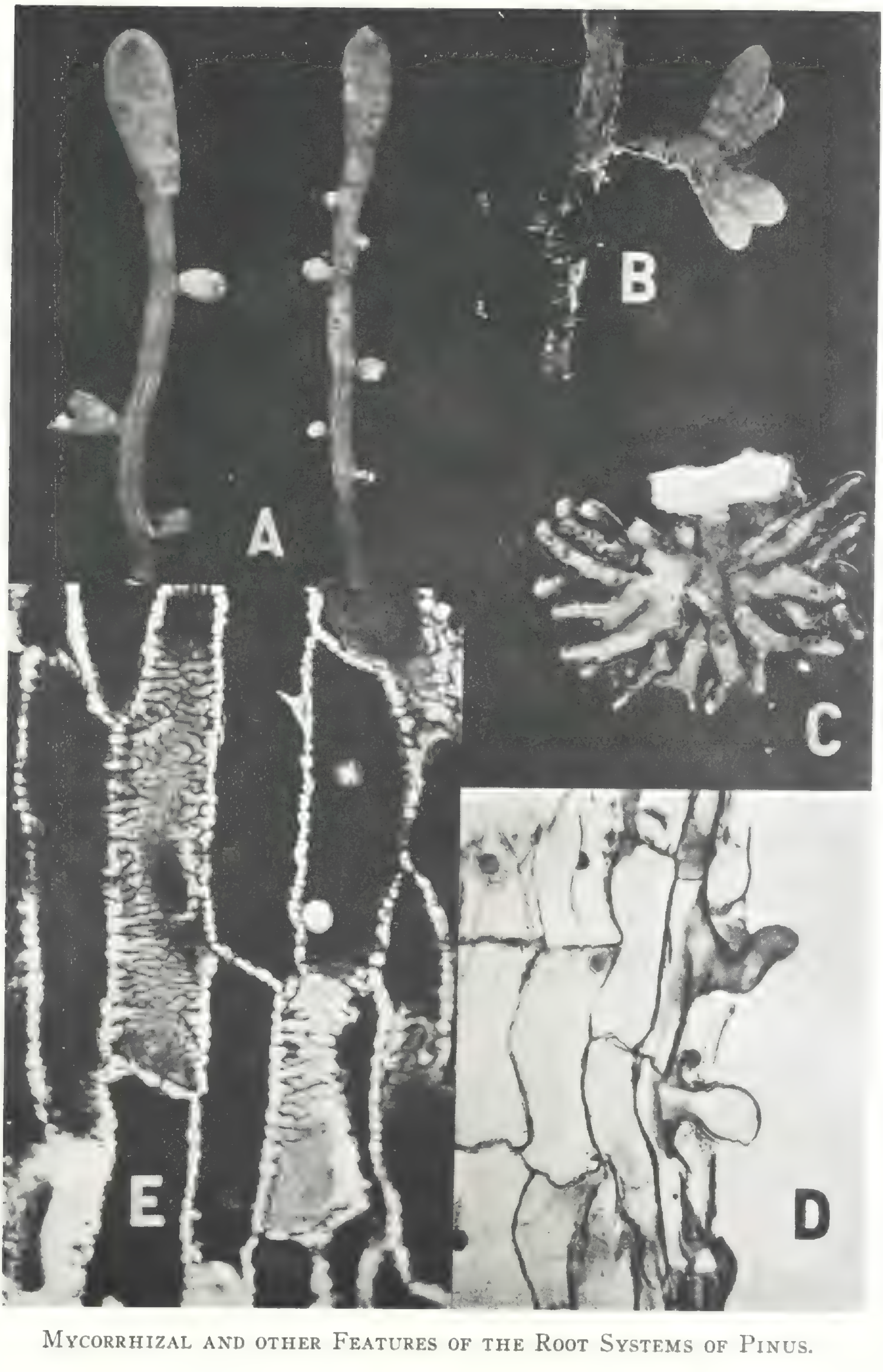
D. Transverse section of monarch short root of *P. sylvestris* grown in open sand culture showing root hairs arising from epidermal cells; × 123. (Nutrient sand culture.)



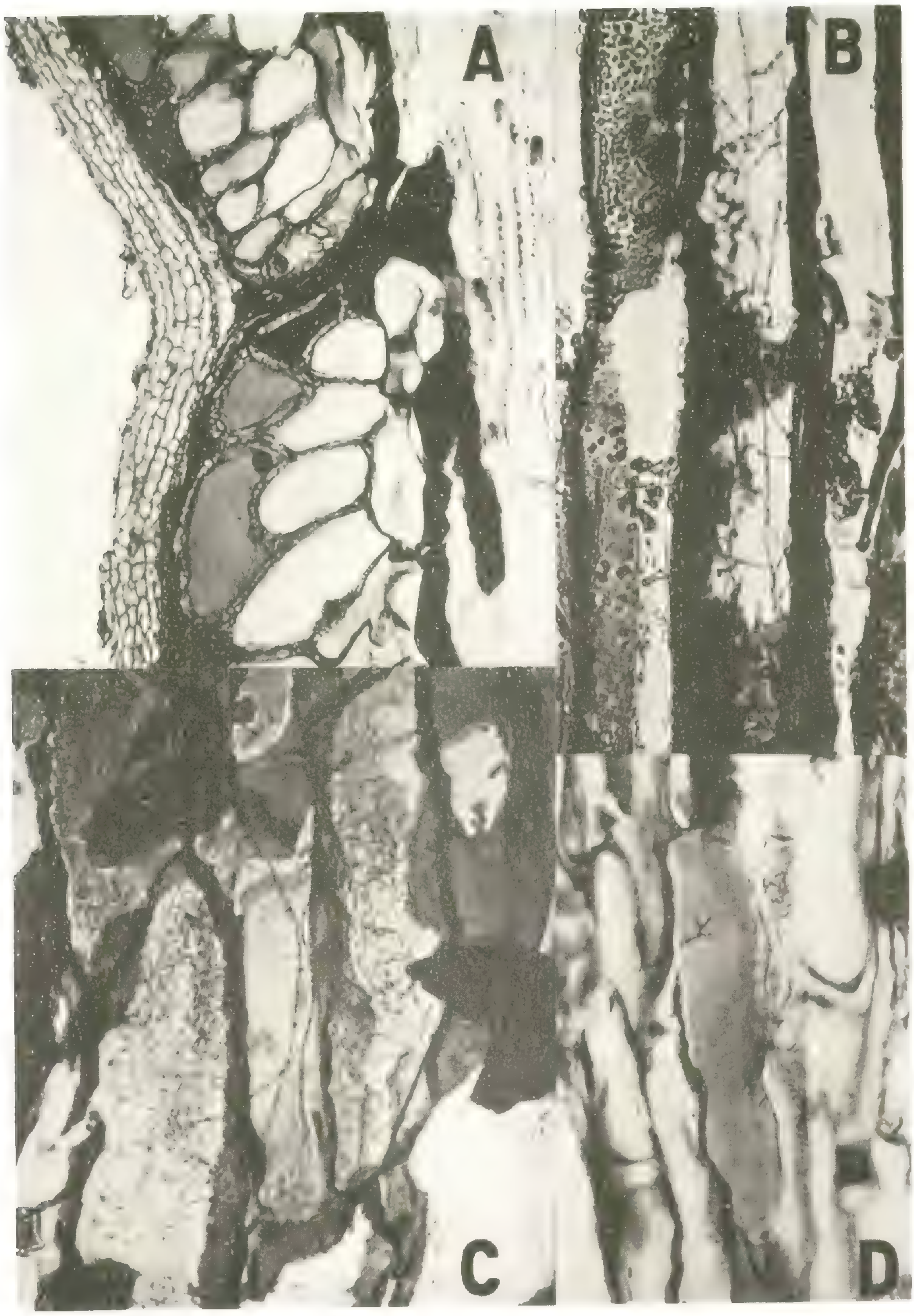
Mycorrhizal and other Features of the Root Systems of Pinus.



MYCORRHIZAL AND OTHER FEATURES OF THE ROOT SYSTEMS OF PINUS.



MYCORRHIZAL AND OTHER FEATURES OF THE ROOT SYSTEMS OF PINUS.



Mycorrhizal and other Features of the Root Systems of Pinus.

Plate 58. A. Pseudomycorrhizal short roots on mother root of *P. sylvestris;* × 12. (Ditto; Plate 57, A; material from unpublished data of Professor E. Melin and the senior author.)
B. Uninfected short roots possessing root hairs on mother root of *P. Strobus* in pure culture; × 12.
C. Mycorrhizal short roots on mother roots of *P. Strobus* grown in pure culture with *Lactarius deliciosus;* × 10.

Plate 59. A. Mycorrhizal mother root tips of *P. Strobus;* plants removed from soil after onset of cold weather, showing also young mycorrhizal short roots; × 8.8. Grown in sand-mixed humus (Infektionsjord from forests in Sweden. See Hesselman, 1927, and Gast, unpublished.)
B. Lateral root initial from radicle of *P. sylvestris* converted into a mycorrhiza; basal portion pseudomycorrhizal; × 12.5.
C. Profusely branched short root of *P. Strobus;* × 8.8.
D. Root hairs on long root of *P. Strobus* originating subepidermally; × 470.
E. Intercellular net (Hartig's net) in a mycorrhiza of *Tsuga canadensis* (L.) Carr.; × 512.

Plate 60. A. Mantle structure and intercellular net of cortical cells in a mycorrhiza of *P. rigida* Mill.; × 330.
B, C and D. Pseudomycorrhizal type of infection in long roots of *P. Strobus;* × 512.

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ADDITIONAL NOTES ON THE ORCHIDS OF THE NEW HEBRIDES AND SANTA CRUZ ISLANDS

OAKES AMES

In an earlier paper[1] on the orchids of the New Hebrides and Santa Cruz Islands, I included all of the species represented in S. F. Kajewski's collections with the exception of several which were too fragmentary for critical study. Since my paper was published additional material has been found and I am now able to complete the record of Kajewski's 1928 and 1929 series. During my studies I have examined a rich collection of specimens containing species that have not yet been reported to be natives of the New Hebrides or Santa Cruz group. In the following paper I have included these, and to make the record more serviceable I have included also a number of species which although already reported from the New Hebrides, are now represented by specimens recently collected or by material referable to them. Unfortunately many of the specimens found by Dr. Morrison on Aneityum lack flowers and are indeterminable. I have only included those species which are quite clear, and in a few cases, when a plant represented a genus new to the region, I have included it, although for want of flowers the specific name could not be arrived at.

For the opportunity to examine the collections made on Malekula, Efate, Eromanga, Aneityum and the Banks Islands by Dr. Morrison and L. Cheeseman, I am indebted to Sir Arthur W. Hill, Director of the Royal Botanic Gardens, Kew.

Habenaria physoplectra Reichenbach f. in Linnaea, XLI. 17 (1876).

Aneityum: Anelgauhat, *Dr. Morrison,* s. n., June 15, 1896; hills between Ithumu and Anelgauhat, *Dr. Morrison,* s. n., July 6, 1896. Efate: Undine Bay, hills, *Dr. Morrison,* s. n., August 27, 1896.

[1]Journal of the Arnold Arboretum, XIII. 127-141 (1932).

The specimens referred to this species are too old to be serviceable for purposes of accurate identification, but vegetatively they bear a close resemblance to Reichenbach's type specimen (*MacGillivray* no. 27) and the withered remains of the flowers on the plant collected between Ithumu and Anelgauhat have the scrotiform spur that is one of the chief characters of *H. physoplectra*. MacGillivray's specimens were collected on Aneityum. Fritz Kraenzlin refers *Habenaria novoebudarum* F. v. Mueller to the synonymy of this species. (Orch. Gen. & Sp. 905).

Habenaria ponerostachys Reichenbach f. in Bonpl. III. 213 (1855).

Aneityum: Hill north of Anelgauhat, *Dr. Morrison*, s. n., June 1896.—Also Philippine Islands.

In habit and floral structure the specimen from Aneityum resembles very closely the type and specimens from Leyte and Mindanao. The broad petals, aristate lateral sepals and the lobes of the labellum are similar to corresponding structures in the Philippine plants. The extension of range is interesting, because *H. ponerostachys* is not common in the Philippine Islands and has not been reported heretofore from any other part of the world.

Habenaria stenodon Reichenbach f. in Linnaea, XLI. 17 (1876).

Banks Islands: Vanua Lava, *L. Cheeseman*, s. n., November 1929.—Already found on Aneityum by MacGillivray.

Corybas mirabilis Schlechter in Fedde, Rep. Spec. Nov. XIX. 22 (1923).

Corysanthes mirabilis Schlechter in Bull. Herb. Boiss. ser. 2, VI. 296 (1906).

Aneityum: *Dr. Morrison*, s. n., June 26, 1896 (dark glossy purple, variegated with whitish lines or meshes on lip and upper sepal).

The type was collected by Dr. Morrison on the summit of the peak between Anumy and Ithug, c. 2300 feet altitude in June 1896. The specimens in the Kew Herbarium are accompanied by a note which reads: "On peak crossed June 26, 1896."

Nervilia sp.

Aneityum: Hill north of Anelgauhat, *Dr. Morrison*, s. n., June 15, 1896.

A single leaf constitutes the specimen examined. This leaf resembles very closely the leaves of *Nervilia MacKinnonii* (Duthie) Schlechter.

Epipogon roseum (D. Don) Lindley in Jour. Linn. Soc. I. 177 (1857).

M a l e k u l a : South West Bay, in bush at sea level, *L. Cheeseman*, s. n., January 1930.—Also Java, Ceylon, New Guinea, tropical India and Australia.

Goodyera triandra Schlechter in Bull. Herb. Boiss. ser. 2, VI. 298 (1906).

E f a t e : Undine Bay, *Dr. Morrison*, s. n., August 20, 1896; Mount Macdonald, *Dr. Morrison*, s. n., August 18, 1896.

The specimens in the Kew Herbarium are similar to the type in being triandrous.

Platylepis Morrisonii Schlechter in Fedde, Rep. Spec. Nov. IX. 161 (1911).

A n e i t y u m : Hills between Anelgauhat and Anumy Valley, *Dr. Morrison*, s. n., June 25, 1896; hills between Ithumu and Anelgauhat, *Dr. Morrison*, s. n., July 6, 1896.

Cystopus aneytyumensis Schlechter in Fedde, Rep. Spec. Nov. IX. 282 (1911).

A n e i t y u m : Near summit of peak south of Ithumu, *Dr. Morrison*, s. n., June 30, 1896; ascent of peak south of Ithumu, 1000-1835 feet altitude, *Dr. Morrison*, s. n., June 30, 1896.

Cystopus fimbriatus J. J. Smith in Bull. Dép. Agric. Indes Néerl. no. 10, p. 3 (1907).

A n e i t y u m : Hills between Ithumu and Anelgauhat, *Dr. Morrison*, s. n., July 6, 1896.—Also Dutch New Guinea.

There are minor differences between the specimens from Aneityum and the type of *C. fimbriatus*, but they are hardly sufficient to justify specific separation.

Zeuxine Erimae Schlechter in Schumann & Lauterbach, Nachtr. Fl. Deutsch. Schutzgeb. 90 (1905).

B a n k s I s l a n d s : Vanua Lava, Avuas, in brush, at 250 feet altitude, *L. Cheeseman*, s. n., October 7, 1929 (roots scarcely lodged in soil).—Also Kaiser-Wilhelmsland.

Anoectochilus sp.

A n e i t y u m : Hills between Ithumu and Anelgauhat, *Dr. Morrison*, s. n., July 6, 1896 (leaves dark green, velvety dull or with sheen according to incidence of light, veined with silvery whitish lines, under surface purplish red veined with greenish).

The only specimens collected are sterile. The leaves resemble those of *Anoectochilus Roxburghii* Lindl.

Vrydagzynea Cheesemanii Ames, sp. nov.

In habitu *V. albidae* Bl. similitudinem gerit. Caules graciles, foliosi.

Folia plus minusve congesta, petiolata, ovato-lanceolata, acuminata, acuta, membranacea. Racemus multiflorus. Bracteae racemi elongatae, lineari-lanceolatae, scariosae, margine glandulosae. Sepala lateralia irregulariter ovato-lanceolata, obtusa, uninervia, apice carinata. Sepalum dorsale anguste ellipticum, obtusum, apice incrassatum. Petala anguste elliptica, obtusa, uninervia, apice incrassata. Labellum in calcar conicum productum; lamina labelli suborbicularis. Columna generis.

Terrestrial herb 17.5-21.5 cm. tall, in facies very similar to *V. albida* Bl. Stems slender bearing several foliaceous bracts near the base, leafy above. Leaves crowded, including the petiole up to 10 cm. long, 1.5-3 cm. wide; lamina ovate-lanceolate, acuminate, acute, papyraceous. Peduncle slender, including the raceme up to 12 cm. long. Raceme 4-5.4 cm. long, about 1 cm. in diameter near the base, tapering gradually upward, densely many-flowered. Bracts of the raceme up to 1 cm. long, linear-lanceolate, glandular-pubescent on the margin, mid-nerve prominent. Ovary about 7 mm. long, smooth. Lateral sepals 3.5 mm. long, 2 mm. wide, asymmetrically ovate-lanceolate, obtuse, conspicuously thickened at the carinate apex, 1-nerved. Dorsal sepal 3.5 mm. long, narrowly elliptical, obtuse, with the blunt apex considerably thickened, 1-nerved. Petals adherent to the dorsal sepal, 3.5 mm. long, 1.5 mm. wide, narrowly elliptical, obtuse, thickened at the tip, 1-nerved. Labellum calcarate, 6 mm. long from the tip of the spur to the tip of the expanded lamina; lamina 1.5 mm. long to the opening of the spur, 2 mm. wide, suborbicular, fleshy, distinctly thickened or bicallose at the base in front of the opening to the spur, somewhat thickened at the tip. Spur about 4.5 mm. long, about 1.5 mm. in diameter, tapering gradually to the obtuse or subacute tip; within on the posterior wall hang two pedicellate verruciform appendages, each appendage and its pedicel 1.5 mm. long. Column 2 mm. long.

M a l e k u l a : South West Bay, at sea level, *L. Cheeseman*, s. n. (type in Herb. Kew; duplicate type in Herb. Ames, no. 37831), January 1930.

From *Vrydagzynea elongata* Bl., this species differs conspicuously in having a conical spur with an acute or nearly acute apex. The smaller flowers, dissimilar lip and larger leaves seem to differentiate it from *V. neo-hibernica* Schltr.

Malaxis lunata (Schltr.) Ames in Jour. Arnold Arb. XIII. 129 (1932).

Microstylis lunata Schlechter in Fedde, Rep. Spec. Nov. IX. 162 (1911).

Aneityum: Hills between Ithumu and Anelgauhat, *Dr. Morrison,* s. n., July 6, 1896; near Anelgauhat, *Dr. Morrison,* s. n., June 1896; ascent of peak south of Ithumu, 1000-1835 feet altitude, *Dr. Morrison,* s. n., June 30, 1896.

Oberonia sp.

Aneityum: Gulley near Anelgauhat, *Dr. Morrison,* s. n., June 13, 1896.

This material collected by Dr. Morrison is in fruit and indeterminable. In general appearance the plant resembles *O. Betchei* Schltr. It also suggests *O. ensiformis* Lindl.

Oberonia sp.

Aneityum: Anelgauhat to Anumy, *Dr. Morrison,* s. n., June 25, 1896.

Sterile specimens that are vegetatively similar to *O. aporophylla* Reichb. f.

Oberonia glandulosa Lindley, Fol. Orch. Oberonia, 6 (1859).

Efate: Undine Bay, *Dr. Morrison,* s. n., August 18, 1896. Aneityum: Hills between Ithumu and Anelgauhat, *Dr. Morrison,* s. n., July 6, 1896 (fruiting specimen).—Also Tahiti.

Liparis elegans Lindley in Wall. Cat. no. 1943 (1828), nomen; Lindley, Gen. & Sp. Orch. 30 (1830).

Aneityum: Peak between Anumy and Ithug, *Dr. Morrison,* s. n., June 26, 1896.—Also Malay Peninsula and Borneo.

Chrysoglossum aneityumense Ames, sp. nov.

Rhizoma gracile, verisimiliter statu juvenili vaginis laxis vestitum. Folium chartaceum, anguste ellipticum, subacutum, basi rotundatum in petiolum sulcatum attenuatum. Scapus erectus, pauciflorus. Sepala lateralia oblongo-lanceolata, acuminata, cum labello saccum scrotiformem formantia. Sepalum dorsale simile. Petala lanceolata, acuta. Labellum conspicue trilobatum; lobi laterales anguste semielliptici, valde obtusi; lobus medius semiorbicularis; discus inaequaliter tricarinatus. Columna crassa, apice alata, basi in pedem producta.

Rhizome slender, partly concealed by the fibrous remains of sheathing bracts, 2-4 mm. thick in dried specimens. Leaf including the petiole 7.5-9.5 cm. long, about 3.5 cm. wide, chartaceous when dry, narrowly elliptical, subacute, rounded at the base; petiole about 1.5 cm. long, terminal on a slender abbreviated pseudobulbous stem. Scape, the continuation of a leafless stem arising from near the base of the leaf, including the few-flowered raceme about 1 dm. long. Raceme 4-6 cm. long, loosely five- to eight-flowered, flowers about 1 cm. apart. Bracts

of the inflorescence about 1 cm. long, lanceolate, acute, membranaceous. Pedicels slender, including the ovary 1.5 cm. long, sharply curved. Lateral sepals 1 cm. long, 4 mm. wide, oblong-lanceolate, acuminate, acute, forming with the labellum a conspicuous scrotiform sac, spreading. Sac 5 mm. long, about 3 mm. in diameter. Dorsal sepal about 1 cm. long, 3.5 mm. wide, similar to the laterals. Petals 9 mm. long, about 3 mm. wide, lanceolate, acute, spreading. Labellum connected with the sac by a narrow claw, including the sac 1.4 cm. long, conspicuously 3-lobed; lateral lobes 4 mm. long, 2 mm. wide, narrowly semi-elliptic, rounded at the apex, separated from the middle lobe by an abbreviated isthmus; middle lobe semi-orbicular, 6 mm. long, 8 mm. wide; disc 3-carinate with the outer carinae auriculate near the base of the lateral lobes and extending nearly to the center of the middle lobe where they become conspicuously elevated into semi-elliptical plates, the central carina is shorter than the lateral ones and hardly expanded at the tip. Column fleshy, free portion 4.5 mm. long, produced at base into a conspicuous foot, at the summit becoming conspicuously winged round the androclinium.

Aneityum: Anelgauhat, below 800 feet altitude, *Dr. R. Morrison,* s. n., June 1896 (type in Herb. Kew; duplicate type in Herb. Ames, no. 39055).

The closest ally of this species appears to be *Chrysoglossum papuanum* (Schltr.) J. J. Smith which differs from the New Hebridean plant in having the lateral lobes of the labellum acute and in having slightly different carinae on the disc. Vegetatively these species are very similar with flowers that are about equal in size.

Dendrobium calcaratum A. Richard, Sert. Astrol. 18. t. 7 (1834).

Aneityum: Hills between Ithumu and Anelgauhat, *Dr. Morrison,* s. n., July 6, 1896; between Anelgauhat and Anumy, *Dr. Morrison,* s. n., June 25, 1896; ascent of peak south of Ithumu, at 1000-1835 feet altitude, *Dr. Morrison,* s. n., June 30, 1896.—Already found on Vanikoro in the Santa Cruz Islands.

Dendrobium gnomus Ames, sp. nov.

Caules dense caespitosi, graciles, perbreves. Folia disticha, linearioblonga, apice inaequaliter bidentata. Racemi pauciflori, laterales. Sepala lateralia mentum obtusum formantia, triangularia, acuta, valde membranacea. Sepalum dorsale lanceolatum, acutum. Petala lanceolata. Labellum elongatum, simplex, infra medium constrictum, bicallosum, supra medium in laminam lanceolatam acutam expansum. Columna generis.

Roots finely fibrous, white, smooth. Stems up to 3.5 cm. long, about

2 mm. in diameter when dry, yellow, deeply furrowed longitudinally, four- to eight-jointed, with the internodes about 5 mm. long and nearly equal in diameter. Leaves alternate, 1.7-2.1 cm. long, 2.5 mm. wide, linear-oblong, tapering gradually toward each end, unequally bidentate at the blunt apex. Leaf-sheaths somewhat complanate, heavily nerved, remaining as loose fibres at the nodes of the stem after the fall of the leaves. Inflorescence breaking forth at the nodes of the naked stems; rachis about 6 mm. long. Bracts of the inflorescence scarious, those subtending the flowers lanceolate, acute. Flowers several, bright purple, about 11 mm. long from the tip of the dorsal sepal to the tip of the mentum formed by the lateral sepals, membranaceous. Lateral sepals slightly spreading, triangular, acute, including the mentum 1 cm. long, about 3 mm. wide near the middle; mentum 4 mm. long, about 2.5 mm. in diameter, closed in front, protuberant anteriorly at the tip. Dorsal sepal lanceolate, acute, 5.5 mm. long, 2 mm. wide. Petals lanceolate, acute, erect, diverging slightly from the dorsal sepal, 5 mm. long, about 1 mm. wide. Labellum 8 mm. long, expanded above the middle into an elliptic-lanceolate lamina which is 2 mm. wide, basal portion narrowly oblong, slightly constricted near the base of the expanded upper portion with a rounded thickening or callus on each side of the constriction. Column produced into an elongated foot.

Santa Cruz Group: Vanikoro, in rain forest at 800 meters altitude, growing in moss, *S. F. Kajewski,* no. 605 (type in Herb. Ames, no. 38083), November 11, 1928 (flowers bright purple, very pretty; (only one specimen seen).

One of the smallest representatives of the Pedilonum section of *Dendrobium,* about equal in size to *D. cyanocentrum* Schltr.

Dendrobium Mohlianum Reichenbach f. in Bot. Zeit. xx. 214 (1862).

Dendrobium neo-ebudanum Schlechter in Bull. Herb. Boiss. ser. 2, vi. 456 (1906).

Aneityum: Between Anumy and Ithug, at 2300 feet altitude, *Dr. Morrison,* s. n., June 26, 1896; hills near Anumy, *Dr. Morrison,* s. n., June 6, 1896. Eromanga: Peak south of Dillon's Bay, *Dr. Morrison,* s. n., August 5, 1896; Traitor's Head, summit of old crater growing on rotten wood, 2400 feet altitude, *L. Cheeseman,* no. 72, August 21, 1930 (flower pale vermillion, not deep).—Also Fiji Islands and Samoa.

Dendrobium Mooreanum Lindley in Jour. Hort. Soc. vi. 272 in footnote (1851).

Dendrobium Fairfaxii Rolfe in Gard. Chron. ser. 3, v. 798 (1889).

Aneityum: Peak south of Ithumu, *Dr. Morrison,* s. n., June

30, 1896 (flower generally pure white, labellum greenish, veined with purple); Anelgauhat to Anumy, *Dr. Morrison,* s. n., June 25, 1896; peak between Anumy and Ithug, *Dr. Morrison,* s. n., June 26, 1896. Efate: Undine Bay, *Dr. Morrison,* s. n., August 27, 1896. Eromanga: Hill south of Dillon's Bay, *Dr. Morrison,* s. n., August 5, 1896.

Dendrobium occultum Ames, sp. nov.

Caules lageniformes vel cylindracei, prope apicem diphylli. Folia oblongo-lanceolata, apiculata, in sicco chartacea. Flores singuli. Sepala lateralia mentum elongatum formantia, oblonga, prope apicem attenuata, apiculata. Sepalum dorsale elliptico-oblongum, apiculatum. Petala oblanceolata, apice rotundata, breviter apiculata. Labellum oblanceolatum, apice apiculatum, prope basim lamella transversa ornatum; discus leviter tricarinatus. Columna generis, clinandrio denticulato.

Stems 1.5-2.5 cm. long, lageniform or cylindrical, bearing at the summit two obliquely ascending leaves, when young concealed by tubular sheaths which persist as stiff erect fibres at the nodes on the mature stems. Leaves 8-13 cm. long, up to 1.5 cm. wide, oblong-lanceolate, apiculate, papyraceous when dry. Flowers 2.8 cm. long, borne singly at the nodes of the leafless stems. Pedicel with the ovary 8 mm. long, ascending. Lateral sepals including the mentum 2.8 cm. long, about 5 mm. wide, oblong, gradually tapering from above the middle to an acute apiculate tip, the nerves somewhat raised on the outer surface in dried specimens, the central one subcarinate near the apex; mentum 8.5 mm. long, slender, 4 mm. in diameter, tapering gradually to the tip where it is about 1 mm. in diameter, closed in front for about one-half of its length. Dorsal sepal 2 cm. long, 5 mm. wide, elliptic-oblong, tapering to an apiculate tip, narrowed toward the base, mid-nerve lightly carinate toward the distal end. Petals about 1.9 cm. long, 7 mm. wide across the upper third, about 2 mm. wide near the base, oblanceolate, rounded at the apex, shortly apiculate, 5-nerved. Labellum about 2 cm. long, about one-third shorter than the lateral sepals, oblanceolate, apiculate, about 6 mm. wide above the middle, the three central nerves more or less prominent, the middle one subcarinate toward the base of the disc; 6 mm. from the base there is a fleshy transverse callus. Column 9 mm. long; clinandrium denticulate.

Santa Cruz Group: Vanikoro, in the moss on rain-forest trees, at 800 meters altitude, *S. F. Kajewski,* no. 604 (type in Herb. Ames, no. 38084), November 11, 1928 (flowers, some cream with yellow-edged labellum, others are purple-pink, but all have the yellow-edged labellum).

Apparently a close ally of *D. asperifolium* J. J. Smith, but the leaves and flowers are not verrucose. In habit similar to *D. pentapterum* Schltr. and *D. Cuthbertsonii* F. v. Muell. The specific name alludes to the tendency of the flowers to be hidden by the moss in which the plants were growing.

Dendrobium purpureum Roxburgh, Fl. Ind. III. 484 (1832).

Dendrobium Morrisonii Schlechter in Bull. Herb. Boiss. ser. 2, VI. 456 (1906).

Aneityum: Hill northeast of Anelgauhat, *Dr. Morrison,* s. n., June 18, 1896; near Anumy, *Dr. Morrison,* s. n., June 1896; hills between Ithumu and Anelgauhat, *Dr. Morrison,* s. n., July 6, 1896 (flowers white).—Also Moluccas.

Dendrobium Quaifei Rolfe, *ined.*

Dendrobium Qnaifei Guillaumin in Bull. Soc. Bot. France, LXIV. 707 (1927), *sphalm.*

New Hebrides: Santo Peak, 4500 feet altitude, *W. T. Quaife,* s. n., May 1903.

The narrowly oblanceolate petals and the dissimilar stelidia of the column separate this species from *D. pseudo-Tokai* Kraenzl. Apparently it is closely allied to *D. montis-yulei* Kraenzl., but it differs sufficiently in the flowers to be regarded as distinct. In habit it is very similar to *D. Mooreanum* Lindl., but from that species it differs conspicuously in having much smaller flowers and a different labellum.

The type consists of a single plant: Caules plus minusve 28 cm. longi, verisimiliter caespitosi, usque ad apicem dilatati, 6 mm. in crassitudine, valde sulcati, flavidi, ad apicem triphylli. Folia plus minusve 9 cm. longa, usque ad 3.3 cm. lata, valde coriacea, apice bidentata. Inflorescentia 20 cm. longa; pedunculus infra racemum plus minusve 11 cm. longus, paucibracteatus. Racemus plus minusve sex-florus, 9 cm. longus. Bracteae racemi vix 5 mm. longae, lanceolatae, concaviusculae, acutae. Pedicelli ascendentes, graciles, plus minusve 3 cm. longi. Sepala lateralia 2 cm. longa, 5 mm. lata, triangulari-lanceolata, acuminata, acuta, nervo medio prominenti. Mentum 8 mm. longum, curvatum, obtusum. Sepalum dorsale simile. Petala 2.8 cm. longa, 7 mm. lata, membranacea, anguste oblanceolata, quinquenervia, obtusa. Labellum plus minusve 1.9 cm. longum, prope medium 1 cm. latum, trilobatum, lobis lateralibus rotundatis, lobo medio 9 mm. longo, 5 mm. lato, triangulari-lanceolato, acuto. Discus supra medium glaber, infra medium callo bisulcato ornatus. Columna generis, stelidiis recurvatis, acutis, terminalibus instructa.

Dendrobium ruginosum Ames, sp. nov.

Caules caespitosi, inferne graciles, prope apicem in pseudobulbum

longitudinaliter ruginosum flavidum producti. Folia ad apicem pseudobulbi congesta, coriacea, elliptico-oblonga vel lanceolata, acuta. Inflorescentia plus minusve quinqueflora. Sepala lateralia mentum obtusum formantia, oblonga, usque ad apicem attenuata, acuta, extus carinata. Sepalum dorsale lanceolatum, apiculatum. Petala prope basim valde angustata, membranacea, supra basim suborbicularia, acuta. Labellum trilobatum. Discus glaber, callo elongato truncato ornatus. Columna generis.

Roots glabrous, whitish, coarsely fibrous. Stems caespitose, 25 cm. tall, seven-jointed; the uppermost internode conspicuously swollen and pseudobulbous, yellow, 7 cm. long, up to 1 cm. in diameter when dry, longitudinally sulcate, tapering to a slender base; the remaining internodes slender, 3.7-4.4 cm. long, 2-3 mm. in diameter, smooth, brownish or yellowish, with the fibrous remains of tubular sheaths persisting at the nodes. Leaves terminal on the pseudobulb, coriaceous, three in number, approximate, elliptic-oblong to oblong-lanceolate, tapering gradually toward the tip, 7.5-8.5 cm. long, 1.7-2 cm. wide, obliquely ascending. Inflorescence longer than the leaves, up to 11 cm. long, rigid, slender, about five-flowered, arising from the axil of a leaf or breaking out from the summit of the pseudobulb below the lowermost leaf. Bracts of the inflorescence about 4 mm. long, lanceolate, much shorter than the pedicels, concave or cymbiform, acute. Pedicels slender, with the verruculose ovary 1.7 cm. long, curving sharply toward the upper end. Flowers rather showy with the sepals and petals white and the labellum pale green with purple stripes. Lateral sepals membranaceous, forming a rounded mentum, including the mentum 2 cm. long, about 5 mm. wide across the middle, oblong, tapering from about the middle toward the acute thickened apex, heavily carinate on the exterior surface along the median nerve, the carina extending beyond the apex of the sepal in a sharp apicule. Mentum 5 mm. long. Dorsal sepal 1.7 cm. long, 7 mm. wide across the middle, lanceolate, apiculate, 5-nerved, lightly carinate on the outer surface. Petals membranaceous, 2.4 cm. long, 1.3-1.5 cm. wide, cuneate at the base, dilated upward into a suborbicular or subrhombic lamina, lightly retuse at the apex with an apicule in the sinus or simply acute, 5-nerved at the base. Labellum about 2 cm. long, 3-lobed; lateral lobes rounded, 1.2 cm. long from the rounded tips to the point of insertion at the base of the labellum, about 5 mm. wide; middle lobe about 7 mm. long, 7 mm. wide, apiculate. Disc smooth, with a heavy truncate median keel on the inner surface which extends to the middle of the labellum. Column about 9 mm. long.

Santa Cruz Group: Vanikoro, in rain-forest at 800

meters altitude, *S. F. Kajewski,* no. 606 (type in Herb. Ames, no. 39056), November 11, 1928 (orchid growing on rain-forest trees; petals white, labellum pale yellow with purple stripes; only two specimens seen).

This species is allied to *D. atroviolaceum* Rolfe, but varies from it in having smaller, differently colored flowers and differently formed petals. The three-lobed labellum serves to distinguish it from *D. Mooreanum* Lindl. and the very dissimilar petals differentiate it from *D. Quaifei* Rolfe, its closest ally in the New Hebrides and Santa Cruz Islands.

Glomera Macdonaldii (Schltr.) Ames, comb. nov.

Glossorrhyncha Macdonaldii Schlechter in Fedde, Rep. Spec. Nov. III. 19 (1906).

Eromanga: Traitor's Head, summit of old crater on mossy trunk of Metrosideros, 2400 feet altitude, *L. Cheeseman;* no. 70, August 21, 1930 (straggling semi-erect branches, flower white).—Already reported from Aneityum.

The type was collected by Macdonald on Aneityum. In the Kew Herbarium there is a sterile specimen collected by Dr. Morrison on Aneityum, between Anelgauhat and Anumy on June 25, 1896. Vegetatively this specimen is very similar to the one from Eromanga collected by Cheeseman.

Phajus amboinensis Blume in Mus. Bot. Lugd.-Bat. II. 180 (1856).

Aneityum: *Dr. Morrison,* s. n., June 22, 1896.—Also Java and Amboina.

This is a small flowered form of the species.

Eulophia macrostachya Lindley, Gen. & Sp. Orch. 183 (1833).

Aneityum: Anelgauhat, on ground under trees, *Dr. Morrison,* s. n., June 9, 1896. Eromanga: Terrestrial, *Dr. Morrison,* s. n., July 18, 1896.—Also Ceylon, Java, Sumatra, Dutch New Guinea and Borneo.

The specimens on which my determination is based have somewhat smaller flowers than usual. Vegetatively the specimens cited above approach *E. novo-ebudae* Kraenzlin in Guillaumin in Bull. Soc. Bot. France, sér. 5, v. 301 (1929), but the labellum is very different if Kraenzlin's description of admittedly poor material is dependable.

Phreatia calcarata J. J. Smith in Bull. Dép. Agric. Indes. Néerl. no. 19, p. 31 (1908).

Banks Islands: Vanua Lava, rain-forest, 3120 feet altitude, *L. Cheeseman,* s. n., October 29, 1929 (flowers white, growing on rotten bole).—Also Papua.

Thrixspermum sp.

Eromanga: Dillon's Bay, *Dr. Morrison*, s. n., August 5, 1896.

There are three specimens, all without flowers, although one is said to have had pale yellow flowers. Vegetatively this species resembles *Thrixspermum Vanoverberghii* Ames, a native of the Philippine Islands.

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THE CHROMOSOME COMPLEMENT OF CYPHOMANDRA BETACEA

THOMAS W. WHITAKER

With six text figures

The genus *Cyphomandra* contains thirty or more species of herbs, shrubs, and small trees, all of which are of South American origin (Bailey, 1925). It is technically distinguished from *Solanum* by the fact that the two cells of the anther are separated by a thickened connective, which appears as a ridge on the back of the anther.

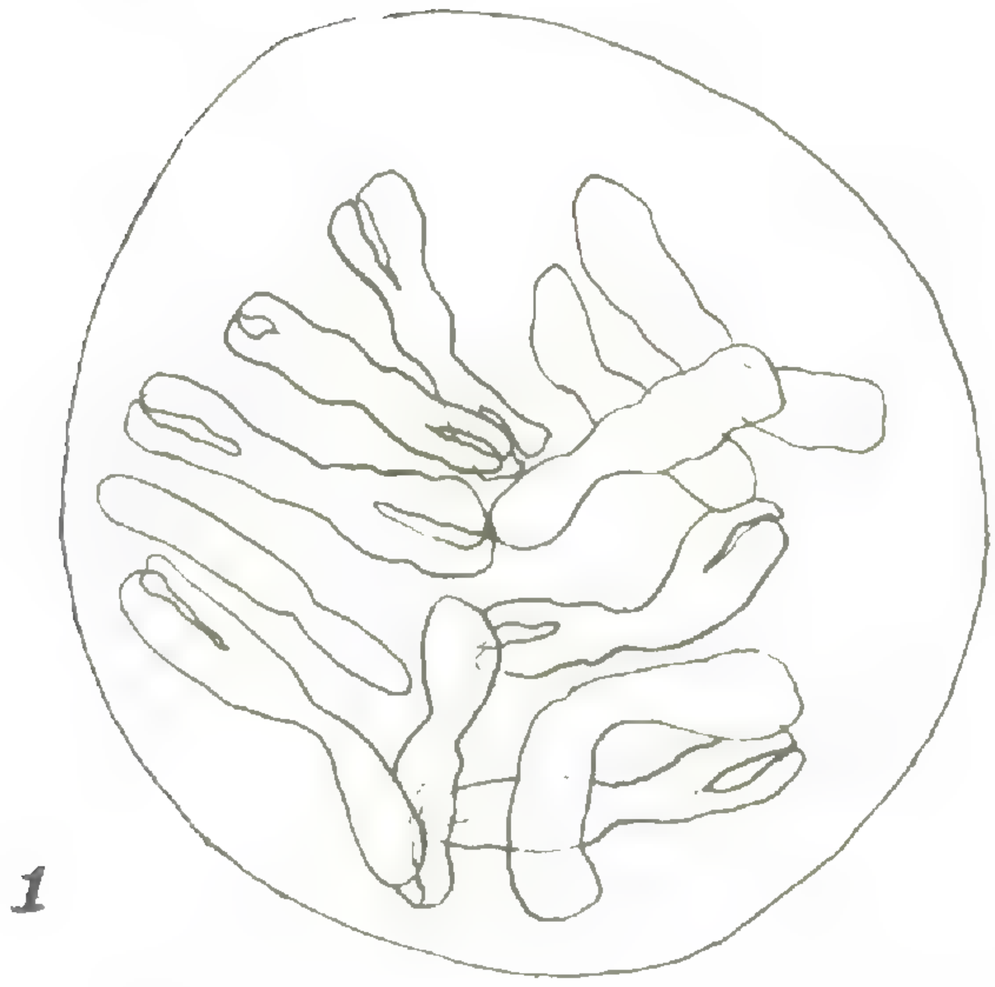
Cyphomandra betacea (Cav.) Sendt., the so-called "Tree Tomato," is cultivated, to a certain extent, by the natives of the American tropics for its edible fruit. It was first described in 1801 under the name *Solanum betaceum* Cavanilles. Later it was placed in the genus *Cyphomandra* by Sendtner (1845).

In the course of an investigation of the chromosome number and behavior of various members of the Solanaceae, several plants of *C. betacea* were made available to the writer through the courtesy of Professor Karl Sax. A preliminary examination indicated that the meiotic divisions were irregular, and, for this reason, it was thought that a further cytological examination would contribute some information on the cause and effect of the irregularities noted at meiosis.

Attention was focused on three points: (1) A description of the nature of the irregularities taking place during meiosis; (2) the effect of these irregularities on the amount of pollen sterility; (3) the probable taxonomic relations of *C. betacea* from a study of its cytology.

Observations on the meiotic divisions were made from aceto-carmine smear preparations. Preparations showing microspore divisions are, as a rule, unsatisfactory when prepared by the usual methods, because the pollen grains are thick and opaque. By fixing with aceto-carmine and exerting considerable pressure on the cover slip, combined with gentle heating of the slide, excellent preparations were secured.

C. betacea has twelve pairs of chromosomes. The haploid complement is shown particularly well at metaphase of the microspore division (Fig. 1). It is apparent that the attachment constrictions are approximately median in all cases, and there are no conspicuous morphological variations among the individuals of the haploid set. In root tip preparations, twenty-four chromosomes are plainly discernible. The somatic chromosomes are characteristically long and slender. Each



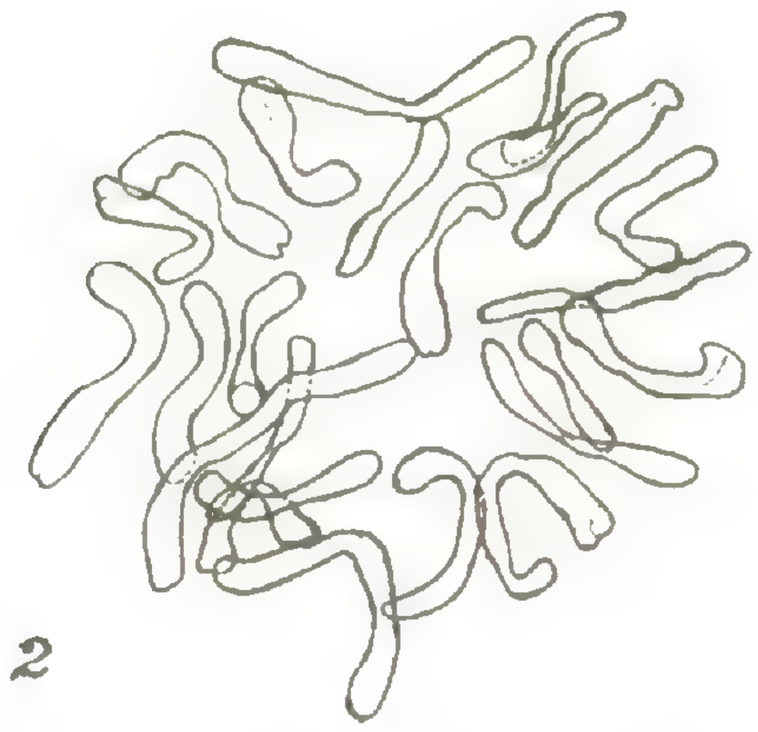
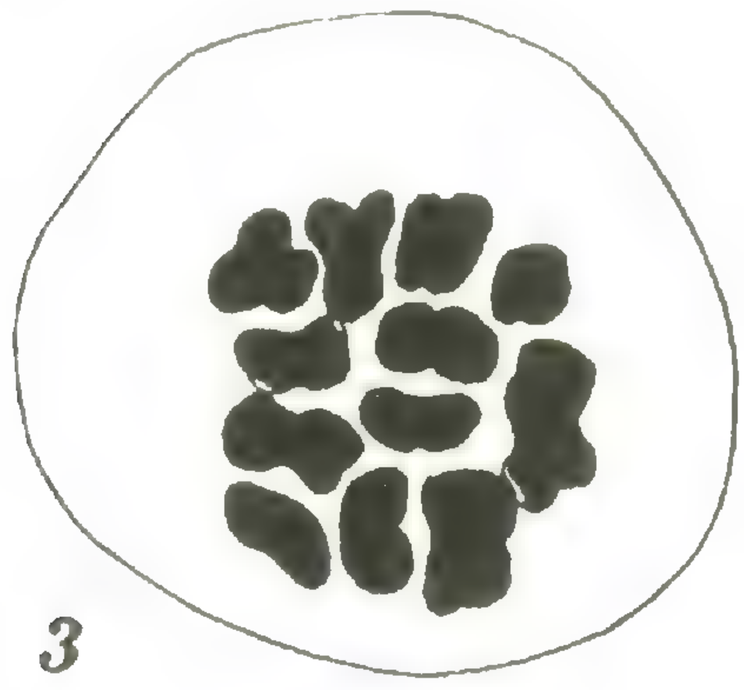
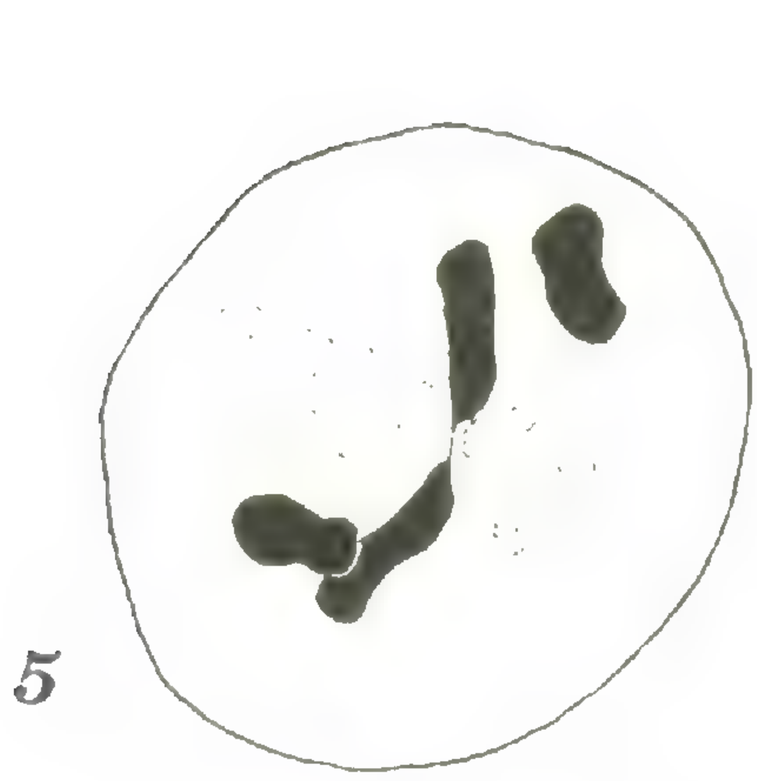


FIGURE 1. THE 12 GAMETIC CHROMOSOMES IN THE FIRST DIVISION OF THE MICROSPORE. — FIGURE 2. THE 24 SOMATIC CHROMOSOMES AT METAPHASE. × 2100.





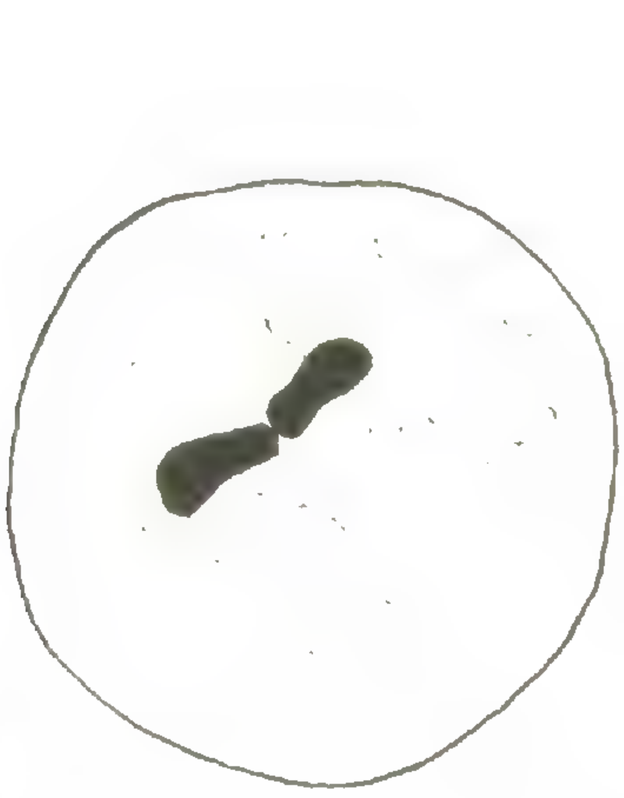


FIGURE 3. THE 12 BIVALENT CHROMOSOMES AT METAPHASE IN THE FIRST DIVISION OF THE POLLEN MOTHER CELL. — FIGURE 4. FIRST METAPHASE SHOWING ONE UNIVALENT AT EACH POLE, THE REMAINDER AT THE EQUATOR. — FIGURE 5. SHOWS THE SAME SITUATION AS IN FIGURE 4 BUT WITH TWO BIVALENTS SHOWING PRECOCIOUS DIVISION. — FIGURE 6. SHOWS A LAGGING PAIR OF CHROMOSOMES. × 2100.

chromosome has a median attachment constriction, and several have secondary constrictions (Fig. 2).

Good figures of diakinesis were difficult to obtain, owing perhaps to the unusual amount of chromatin contained in the relatively small nucleus, but where satisfactory figures were examined, it is clear that two of the bivalents have only one terminal chiasma. The remaining bivalents have at least two chiasmata and, in certain cases, as many as three chiasmata.

The structure of the individual chromosomes is very clear at metaphase of the first meiotic division, and the spiral chromonemata are easily visible. At this point several abnormalities were observed to interrupt the ordinary sequence of the meiotic process. Briefly these abnormalities were: (1) One and sometimes two of the univalents were located at each pole, while the remainder of the set were still at the equator (Fig. 4); (2) two pairs of chromosomes frequently showed precocious division (Fig. 5); (3) lagging of one pair of chromosomes was repeatedly observed (Fig. 6). It is estimated that these abnormalities occurred in about 50% of the cases.

The irregularities observed at meiosis are reflected in the pollen, since fully 25% of the pollen grains are aborted. The percentage of aborted pollen in some flowers is much greater, reaching an extreme limit of 50% pollen sterility.

DISCUSSION

On comparing the size of the metaphase chromosomes of *C. betacea* with those of *Solanum Capsicastrum* (arbitrarily selecting *S. Capsicastrum* as a representative of the other Solanaceae), it was found that the chromosomes of *C. betacea* averaged slightly over twice as long as those of *S. Capsicastrum* in similar preparations at the same stage. This same relationship existed regarding the width of the chromosomes. The diameters of the pollen mother cells of *C. betacea* and *S. Capsicastrum* stand in the ratio of 1:1.33. Thus the volume of the pollen mother cells of *C. betacea* is approximately three times that of *S. Capsicastrum*.

Vilmorin and Simonet (1928) have reported the chromosome number of a considerable range of solanaceous plants. In their studies they have found an arborescent species of *Solanum* (*S. glaucum*) whose chromosome complement consists of twelve large, irregularly shaped chromosomes. This is the only species of the Solanaceae yet reported where the individual chromosomes approach in size those of *C. betacea*.

Aside from the striking difference in the size of the chromosomes and pollen mother cells, *C. betacea* is very similar, cytologically, to other

members of the Solanaceae. The closely related genera *Salpichroa*, *Solanum*, and *Lycopersicum* all have a basic number of twelve chromosomes and ring bivalents. In this connection it is interesting to note that although the individual chromosomes of *C. betacea* are considerably larger, both in width and in length than those of other species of Solanaceae, the type of association is identical with that found in species with smaller chromosomes.

The amount of pollen sterility (25%) found in *C. betacea* is quite unusual in a pure species, although by no means exceptional. Professor Jack, of the Arnold Arboretum, informs me that *C. betacea* is considered a good species and that it is quite unlikely that it could have hybridized with any other member of the genus, as it is very distinct from other species of *Cyphomandra*. Therefore the irregularities at meiosis and high pollen sterility cannot be accounted for by assuming that the plants under consideration were a direct result of hybridization with a closely related species.

It has been suggested that the irregularities under consideration might be due to the fact that the plants were grown under a lower temperature than that to which they are accustomed in their native habitat. Acting on this suggestion, the writer has examined pollen of plants from Cuba. (This pollen was supplied through the kindly cooperation of Mr. R. M. Grey of the Atkins Institution of the Arnold Arboretum.) Pollen sterility of the Cuban plants averages approximately 5%, compared with an average sterility of 25% of plants flowering at the Bussey Institution. It appears as if this considerable difference in pollen sterility is significant, particularly since the pollen sterility was as high as 50% in a few cases of the plants grown under different environmental conditions.

There is good evidence that meiosis in plants is, in some measure, disturbed by lowering of the temperature. Belling (1925) first suggested that pairing of the chromosomes might be more easily disturbed by cold in tropical or subtropical plants than would be the case with plants of temperate regions. He has also suggested that cold treatment might be an important means of inducing tetraploidy in tropical plants. Sax (1931) kept plants of *Rhoeo discolor* at about 45 degrees Fahrenheit for several days. The pollen mother cells of these plants showed a complete lack of pairing of the chromosomes at the first meiotic division. When the cold treated plants were again placed under normal conditions, the ordinary meiotic behavior was restored.

Since the plants of *C. betacea* were not critically tested in a similar manner to find out if the temperature was the factor responsible for the

pollen sterility observed, it is impossible to state with certainty that temperature was the environmental variable.

However, what little evidence available is highly suggestive and indicates that the irregularities during meiosis are directly traceable to the low temperatures prevailing when the plants were examined. The manner in which temperature seems to operate, in this case, is by reducing the number of chiasmata in certain chromosomes, which results in very loose pairing at diakinesis.

This naturally leads to speculation in regard to the way in which plants cope with conditions of decreasing temperature. From the evidence at hand, it seems as though the answer to this question may be that decreasing temperature causes greater variability, which, in turn, increases the chances of a mutant's being produced that would be better able to survive under the new set of conditions.

SUMMARY

1. *Cyphomandra betacea* has twelve pairs of chromosomes. The chromosomes of this species are considerably larger than those of any solanaceous plant yet reported.

2. The irregularities which occur during meiosis are discussed, and evidence is brought forth to indicate that these irregularities were due to the temperature conditions prevailing at the time the plants were in flower.

3. Pollen sterility averaging about 25% has been found in the plants examined. This value, when compared with 5% pollen sterility found in plants grown in Cuba, is considered significant and may be attributed to the difference in temperature under which the plants were grown.

4. The indications are that temperature operates on the meiotic division by reducing the number of chiasmata per bivalent in certain chromosomes.

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STUDIES ON THE PRECIPITIN REACTION IN PLANTS
III. A BIOCHEMICAL ANALYSIS OF THE "NORMAL PRECIPITIN REACTION"[1]

KENNETH S. CHESTER AND THOMAS W. WHITAKER

With four text figures

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[1]For parts I and II see Vol. XIII. 52-80 and 285-296.

I. INTRODUCTION

Thirty years ago Marshall Ward concluded his researches on the parasitism of fungi (*22*) with the words: "Immunity depends entirely upon the physiological reactions of the protoplasm of the fungus and the cells of the host. In other words, infection and resistance to infection depend on the power of the fungus protoplasm to overcome the resistance of the cells of the host by means of enzymes or toxins, and reciprocally on that of the protoplasm of the cells of the host to form anti-bodies which destroy such enzymes or toxins." This statement, at the time little more than a shrewd conjecture, inaugurated an epoch of new conceptions in phytopathology and physiology. Soon there appeared the classic studies of Bernard in which it was shown that the symbiosis between the orchid and its mycorrhizal partner is not the static, passive equilibrium which it had heretofore been considered, but that it is an active, dynamic parasitism in which the orchid host controls through the mechanism of specific internal prophylaxis the erstwhile parasitic invader, that it is in the words of Gäumann "a parasitism with the brakes on" ("ein abgebremster Parasitismus") (9).

Long before this time, however, the conception of a dynamic internal prophylaxis had served to inspire fruitful researches in human medicine. Ward's analogy was but an echo of the doctrine of acquired immunity in animals. It was thus necessarily resultant that the unexperienced phyto-immunologist should look for guidance to his more experienced colleagues in the field of human immunology. Phyto-immunology has assuredly profited from this new application of medical experience, but there is a grave danger in attempting to transplant *in toto* the techniques and conclusions of one branch of science to the very different soil of a kindred science. There is an unavoidable tendency to interpret *analogies* as *homologies*, failing, however, to consider the inherent differences which characterize the materials of the two sciences.

As early as 1902 (11, 16) phenomena in plants resembling immunological behavior in animals had been observed. Plant saps were found to act toward animal proteins in a fashion resembling that of immune blood in the presence of its specific antigen; they agglutinated and hemolyzed blood corpuscles and precipitated foreign proteins. In succeeding years the literature has become replete with similar observations and in almost all cases the terminology and interpretations of human medicine have been bodily appropriated in the establishment of the science of plant immunity. As the most striking and pertinent example of such non-critical appropriation of zoöimmunitary concep-

tions must be cited the researches dealing with the reactions of grafted plants. The work of Kostoff (12) has already been discussed in this series of studies. It is sufficient to state that this worker found in studying the *Solanaceae* that aqueous extracts of certain plants precipitated *in vitro* in the presence of other such extracts, that after grafting two such plants together the intensity of the precipitation sometimes increased, that in certain cases the overlayering of two extracts resulted in a clear zone at the plane of contact, that the plastids of grafted plants sometimes showed a clumping together in masses, and that various malformations and abnormalities accompanied these other phenomena. All of this, without reserve, was interpreted in the terms of antibody formation: the precipitating action was due to "precipitins" (which term has a very specific meaning in zoöimmunity); the clear zones resulted from the activity of "lysins"; the clumping of the plastids was an "agglutination." Kostoff's work has been accepted as a most conclusive link in the chain of proof that plants may elaborate antibodies of the zoöimmunitary type (2, 8). Indeed one worker, Silberschmidt (21), has published a long and painstaking critique of Kostoff's work suggesting numerous modifications based entirely upon the conception that the reactions observed are of the nature of immunological reactions in animals, although Silberschmidt recognizes in the display of reactions certain differences from the reactions of animal immunology.

It is manifest that if the reactions observed in the *Solanaceae* are *homologous* with the zoöimmunological reactions then a most important step has been taken in the demonstration of antibody formation in plants. If, on the other hand, the reactions observed in the *Solanaceae* are only *analogous* to those in serology, if they are not characterized by the same properties and are not of the same significance as are the reactions in animal immunology, then it is of paramount importance to point out their true significance, be it immunological or merely biochemical in its more limited sense. For this reason the present study was undertaken, in order to determine the nature of the reactions heretofore described as immunological. A short preliminary account of this analysis has already been published (5), and it is the purpose of the present study to substantiate the preliminary account with experimental data.

II. MATERIALS AND METHODS

The plants employed in this study were of species from a variety of herbaceous and woody families; they are enumerated below:

LEGUMINOSAE: *Robinia fertilis* Ashe.

OLEACEAE: *Ligustrum ibota* Sieb. & Zucc., *L. obtusifolium* Sieb. & Zucc., *L. vulgare* L.; *Syringa vulgaris* L.
PLATANACEAE: *Platanus acerifolia* Willd.
ROSACEAE: *Prunus Armeniaca* L. var. *ansu* Maxim. and var. *"Mikado"* Hort.
SAXIFRAGACEAE: *Ribes Carrierei* Schneid. (*R. glutinosum albidum* Jancz. × *R. nigrum* L.); *Hydrangea paniculata* Sieb. var. *grandiflora* Sieb.
SOLANACEAE: *Atropa Belladonna* L.; *Browallia viscosa* HBK.; *Capsicum frutescens* L.; *Cyphomandra betacea* Sendt.; *Datura ferox* L., *D. innoxia* Mill., *D. metel* L., *D. Wrightii* Hort. = *D. meteloides* DC.); *Lycopersicum cerasiforme* Dun.; *Nicotiana alata* Lk. & Otto, *N. acuminata* Grah., *N. Cavanillesii* Dun., *N. glauca* Grah., *N. glutinosa* L., *N. Langsdorffii* Weinm., *N. nudicaulis* Hort., *N. Palmeri* Gray, *N. paniculata* L., *N. plumbaginifolia* Viv., *N. Rusbyi* Britt., *N. rustica* L., *N. Sanderae* Hort. Sand. (*N. alata* × *Forgetiana*), *N. suaveolens* Lehm., *N. sylvestris* Speg. & Comes, *N. Tabacum* L., *N. tomentosa* R. & P., *N. trigonophylla* Dun.; *Petunia violacea* Lindl.; *Physalis peruviana* L.; *Salpiglossis sinuata* Ruiz & Pav.; *Solanum Capsicastrum* Link, *S. Melongena* L., *S. nigrum* L., *S. tuberosum* L.

The Solanaceae enumerated were greenhouse plants for the use of which the writers are indebted to Professor E. M. East. The woody plants were mature specimens in the ornamental collection of the Arnold Arboretum. Leaves from the woody species were collected on Sept. 2, 1931, dried at room temperature for one month, and then pulverized and stored in wide-mouthed glass bottles until used. The solanaceous leaves were collected as needed. In nearly all cases reported below the solanaceous extracts were made from leaves which had been dried in a dessicating oven at 55°C. for from one to three days and then pulverized.

The **method of extraction** varied according to the requirements of the various experiments. It is necessary to distinguish here between pre-extraction and solution. The procedure of pre-extraction and its significance in protein reactions has been thoroughly discussed by Silberschmidt (21). Briefly the purpose of a pre-extraction process is to remove from the tissues substances, chiefly lipoids, which give non-protein reactions and hence tend to render the readings inaccurate. Pre-extraction usually involves extraction with strong alcohol, ether, and chloroform. Such pre-extraction was employed in certain of these experiments to determine its effect upon the resulting reactions (see pages 000 and 000), but was not used in the remainder of the experiments because it was the purpose of these experiments to determine the reactive constituents of extracts of the type used by Kostoff, in which no pre-extraction was employed.

The leaf powders or pulps were then extracted in the solvent designed to dissolve the reactive principles. In most of the experiments reported below distilled water was used as a solvent. Physiological NaCl solution was used in some cases in place of the water, particularly in certain of the dialysis experiments in which it was desirable to avoid the autoprecipitation of extracts due to the withdrawal of electrolytes. Here, too, in certain experiments to determine the relative value of various solvents, a selection of several processes was employed, but, unless otherwise stated in the experiments to follow, the reader may understand that distilled water was used as a solvent. The concentration customarily employed was one part by weight of fresh leaf pulp to two parts of solvent or one part dried leaf powder to ten parts of solvent. This concentration is here referred to as normal concentration (N) and further dilution is indicated by the appropriate fraction (N/2, etc.). Extraction of reactive substances occupied two hours at room temperature or 18 hrs. at 2°C. in all cases, it having been previously ascertained that there is no significant difference in the reactivity of extracts prepared under such conditions.

The **clarification** of the extracts was accomplished by various means depending upon the difficulty in obtaining clear solutions. The dried leaves yielded clear extracts much more readily than fresh leaves, and on the whole little difficulty was experienced in obtaining crystal-clear extracts. The customary procedure was to extract in an open beaker or large test-tube, stirring from time to time, to pour the pulp and extract into a funnel lined with a fine filter paper (C. S. & S. #589) which in turn was lined with sterile gauze. The gauze was then drawn together to form a bag, the liquid squeezed out into the filter paper, and the clear filtrate used in testing. In a few cases it was necessary to repeat filtration through one or two other papers. When dealing with a very small quantity of tissue (we have been able successfully to extract .1 gm. of tissue in 1 cc. of water) it was customary to centrifuge, rather than filter, drawing the clear supernatant liquid off into another tube with a fine pipette. In only a few cases was it impossible to obtain clear extracts. *Salpiglossis*, *Browallia*, and *Hydrangea* gave the most trouble in this connection, and when it was necessary to use extracts from such species the procedure employed was to catch the filtrate immediately it came through the filter and to test it at once. In any case these extracts were used in very few of the experiments reported below. *Nicotiana Rusbyi* proved to be rather refractory in aqueous solution but where physiological saline was used as a solvent, the extracts were satisfactory. On standing for several days, even at

2°C., there was frequently an autoprecipitation of the extracts, but in no case of the many tested did the subsequently cleared extracts exhibit an altered reactivity. Extracts which were to be preserved for any appreciable amount of time were covered with ¼"-½" of toluol and kept in the cold. Bacterial or fungus contamination rarely occurred and then only in extracts which had stood for a long period in the cold. Although it was ascertained that such contamination did not affect the reactivity of the extracts (see page 000) such extracts were not used in the tests reported below unless specifically so stated.

For **testing,** the procedure described in an earlier paper (4) was employed. With a fine pipette .2 cc. of the liquid of greater density was introduced into the usual small test tubes and over this was layered an equal quantity of the second liquid to be tested. The length of time of reading varied with the requirements of the experiments from 5 to 40 minutes. Where gross results were desired a duration of 5-10 minutes was satisfactory. In fact the reactions observed were usually so marked that a maximum reading could be made in a very few minutes, while a continuation of the time merely permitted the precipitates to diffuse through the extracts making readings less accurate. In a few of the experiments where only gross differences were of importance, the tests were performed by mixing equal quantities of the two extracts in 3" test tubes and observing the resulting cloudiness in comparison with the unmixed liquids. Throughout this paper, in any case, each experiment is a unit, performed under homogeneous conditions and for the purpose of demonstrating one point. The whole series of experiments were not devised for numerical comparison with each other, and accordingly the demands of scientific caution have been met by performing each experiment under its own set of standard conditions. The numerical value assigned to any given reading was according to one of two scales. The first scale, and that employed in nearly all cases, is that described and figured in an earlier paper of this series (4) in which a trace of a reaction (t) is succeeded by reactions of strength 1, 2, 3, and 4 (maximum ever observed). A more accurate measure of reactivity which was employed in a number of cases was to calculate the numerical sum of the readings (according to the first scale) at 1, 5, 10, 20, 30, and 40 minutes respectively. To be sure neither scale is wholly free from error, and both are arbitrary measures; but it is the belief of the writers that the readings within the limits of these experiments are accurate within the range of 1 unit on the t-1-2-3-4 scale and that a greater accuracy of reading is not compatible within the inherent variability of the material and technique. All of

the readings reported in this paper were made by the same observer, a second observer being consulted in case of doubt.

Numerous **controls** were employed in each experiment. The extracts were tested against the pure solvents and against such other fluids as the individual experiments required.

Protein determinations by the use of the Millon and xanthoproteic tests were resorted to, in general, only in the dialysis experiments and then only for the purpose of obtaining a measure of the efficiency of the dialyzing membranes. Silberschmidt (21) feels that such determinations should be made on all extracts. When it is possible to say with assurance that one is dealing with protein reactions of the zoöimmunitary type, then the protein tests will be of value, but since the aim of the present study was to determine the nature, protein or otherwise, of the reactive substances, it was not felt necessary to employ protein tests in all cases. Similarly other tests (for chloride, oxalate, calcium, etc.) were employed only as the specific experiments required them.

Since a brief summary of most of these results has already been published (5) it is possible to state at the outset the sequence of points which are to be considered in the present paper. It was pointed out in this earlier summary that a reaction responsible for the majority of positive tests which have been observed is the reaction between calcium and oxalate ions in two respective extracts. It then being possible to eliminate from discussion the calcium oxalate reaction, attention may be focussed on the remaining reactions. Having ascertained that there are at least three other such reactions, designated here respectively as the AB, MN, and XY reactions, and their respective properties having been determined, it is finally possible to analyze the experimental data of Kostoff, Silberschmidt, East and Chester with respect to the rôle of these various reactions in the published accounts of the "normal precipitin reaction," and to point out the significance, immunological or otherwise, of the precipitin reaction in plants. Hence the remainder of this paper will concern itself with answering the following questions: 1. What is the nature of the reactions occurring between *Platanus*, *Robinia*, *Ribes*, and *Prunus?* 2. What is the evidence supporting the statement that a calcium oxalate reaction is responsible for the majority of the recorded positive precipitations? 3. What proof is there as to the presence and number of other reactions in the plants studied? 4. What are the properties of these additional reactions? 5. What light does this study cast upon the previously recorded studies of the precipitin reaction in plants? 6. What is the theoretical and practical significance of the precipitin reaction in plants?

III. THE NATURE OF THE REACTIONS OCCURRING BETWEEN PRUNUS, PLATANUS, ROBINIA, AND RIBES

Early in this study the question was considered whether or not the precipitations observed were due to inorganic constituents. Attention was diverted from the theory that they might be explainable in such simple terms because of the following facts: (a) the specificity exhibited by the reaction (4) appeared hardly in conformity with such a simple explanation; (b) accepting Kostoff's work at face value it would be inconceivable from the complexity of his results and their apparent immunological significance that he was dealing with such a simple reaction; (c) when in one preliminary experiment extracts of *Platanus* and *Prunus* were ignited at red heat and then the ash redissolved in water, the reaction had disappeared. The influence of the immunological school of thought was so strong that the writers as well as their various associates were strongly inclined to believe the reactions highly complex in nature. The series of experiments devised accordingly emphasized the proof of the presence or absence of protein in the reactive fractions of the extracts. With the elimination of protein as a reactive principle, attention was directed to the other complex chemical constituents of the extracts, and not until most of the major chemical groups had been eliminated was attention forcibly drawn to the function of the inorganic constituents. The evidence is accordingly of two sorts, negative evidence that the proteins and various other chemical groups could not be responsible for the reactions, and positive evidence that calcium-oxalate was responsible. The present section will treat with the first of these subjects, i. e. the negative evidence, while the following section will deal with the proof of the calcium oxalate reaction.

The first experiments performed were with the species of woody plants enumerated above, but particularly with *Prunus Armeniaca* "*Mikado*," *Platanus acerifolia*, *Ribes Carrierei*, and *Robinia fertilis*. Later the results were extended to include the other species listed above. Of the extracts employed the *Prunus* tests strongly against *Platanus*, *Robinia* and *Ribes*, while the latter three are negative to each other.

A. Effect of Salt Concentration on the Reactions

As a primary step in the investigation of the nature of these reactions it was felt desirable to study the effect of certain physical factors on the reaction, in order to determine the optimum conditions for reaction, to observe the specific effect of variation of such factors, and to obtain a standard technique for subsequent work. The following experiment

was therefore first set up for the purpose of studying the effect of the variation of the electrolytic concentration in the extracts.

Five samples of dry leaf powder of *Platanus*, *Ribes*, and *Robinia* respectively were extracted in the usual manner in ten times the weight of solvent. For solvents were used a series of phosphate buffer solutions prepared according to the system worked out by Cohn (6). All the buffers had the same pH, namely 6.0 (which is relatively close to the normal pH of these extracts). On the other hand the five buffers of each of the three series had salt concentrations respectively of .06M, .12M, .30M, .60M, and 1.2M. This system of buffers has the advantage of introducing only one metallic ion [KH_2PO_4 (acidic) : K_2HPO_4 (basic)]. The fifteen extracts were then cleared and tested against one

TABLE I.

EFFECT ON THE PLATANUS-ROBINIA-PRUNUS PRECIPITIN REACTION OF VARYING THE SALT CONCENTRATION.

		Robinia...Phosphate content (Cohn)						Platanus...Phosphate content (Cohn)					
		.06 M	.12 M	.30 M	.60 M	1.2 M	Total	.06 M	.12 M	.30 M	.60 M	1.2 M	Total
	.06 M	15.0	5.0	5.5	3.0	2.5	31.0	15.0	14.5	9.5	4.0	0.0	43.0
Prunus	.12 M	11.0	9.5	10.0	9.5	4.5	44.5	17.0	15.0	8.0	5.5	2.0	47.5
phosphate content	.30 M	11.0	11.0	6.0	7.0	5.5	40.5	16.0	15.0	7.5	4.0	3.0	45.5
	.60 M	11.0	10.5	9.5	4.0	3.0	38.0	17.0	15.0	9.5	2.0	0.5	44.0
	1.20 M	15.0	9.5	4.5	2.5	2.5	34.0	15.0	12.5	4.5	0.0	0.0	32.0
	Totals	63.0	45.5	35.5	26.0	18.0		80.0	72.0	39.0	15.5	5.5	

another. The results of these tests are given in Table I. The numerical values assigned to the various reactions were computed by summating the strengths of reaction at the various time intervals from 1 to 40 minutes. For control experiments the various extracts of *Robinia* were tested against one another in all possible combinations; the same was done with the extracts of *Prunus* and *Platanus*. All extracts were tested against the various strengths of pure buffer, and finally the *Robinia* series was tested against the *Platanus* series. All of these control tests were completely negative.

Several points are brought out by this table. In the first place one observes that although *Robinia* and *Platanus* behave in the same fashion with regard to salt concentration, both differ in marked fashion from *Prunus*. Variation of salt content through this wide range hardly affects *Prunus* while *Robinia* and *Platanus* show a steady decline in reactivity as the strength of salt increases. This fact would indicate that the reactive substances in the two types of extract are different, a

situation not in conformity with that in animal immunology. Since the higher concentrations of salts used in this experiment are far greater than occur in ordinary water or saline extracts, this experiment also reveals that with regard to salt concentration the conditions under which the experiments have previously been performed have been optimal. And finally the relative insensitiveness, at least of *Prunus*, to salt concentration is not in accordance with the great dependence of serological reactions upon salt concentration, and affords a first suggestion as to the non-protein nature of the reaction.

B. Effect of Hydrogen ion Concentration on the Reactions

This first experiment having revealed that the reaction is apparently not affected by the weaker salt concentrations used, and having indicated the best salt concentrations for further study, the next step was to vary the pH while holding the salt concentration near the optimum. For this purpose *Robinia* and *Prunus* were chosen. Each was divided into 9 samples and each series of samples was extracted in the usual manner in a series of buffers (Cohn's system) all having the same salt concentration (.06M) but having a variety of hydrogen ion concentrations. The range of pH varied from 5.2 to 8.4, since this is the maximum range possible with Cohn's system. However, it must be emphasized that the extreme values here used (5.2 and 8.4) with this buffer system are susceptible to a somewhat greater error than the remaining values. This pH range extends from a point somewhat more acid than the ordinary pH of the extracts (except *Prunus* which may fall as low as 4.0-4.5) to a point far more alkaline than has been observed in any normal extract. The extracts having been prepared, all the possible combinations were made in precipitin tubes and as in the preceding experiment the readings were made at intervals for 40 minutes and then summated. The results of this experiment are shown in Table II.

The results of this experiment are of interest in confirming the earlier findings of Kostoff that the reaction bears no relation to pH of the extracts. It is seen from a study of the table that no significant difference is to be observed throughout the whole of the pH range studied. This does not directly afford evidence as to the nature of the reaction, since the precipitin reaction in animals is also independent of pH within reasonable limits (17) but it does indicate that there is no necessity for a careful checking of the pH of every experimental series.

As controls for this experiment each member of both series was tested against the complete series of pure buffers, the reactions in all cases being negative.

A rather interesting phenomenon was observed in connection with this experiment, namely that variation of pH in such a graded series revealed that the plant extracts thus prepared contain coloring substances with the behavior of chemical indicators. In both *Prunus* and *Robinia* the pH series showed a progressive darkening of color as one passed from the acid to the alkaline extremes. In *Prunus* the color ranged from pale lemon yellow at 5.2 to molasses brown at 8.4. The *Robinia* extracts were all lighter than those of *Prunus* and ranged from

TABLE II.

EFFECT ON THE PRUNUS-ROBINIA PRECIPITIN REACTION OF VARYING THE pH OF THE SOLVENT

		Robinia...pH									
		5.2	5.6	6.0	6.4	6.8	7.2	7.6	8.0	8.4	Total
Prunus pH	5.2	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	126.0
	5.6	17.0	17.0	17.0	17.0	17.0	17.0	15.0	12.5	12.5	142.0
	6.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	15.0	15.0	149.0
	6.4	17.0	17.0	17.0	17.0	17.0	14.0	14.0	14.0	14.0	137.0
	6.8	17.0	17.0	17.0	16.0	16.0	16.0	15.0	15.0	15.0	140.0
	7.2	17.0	17.0	17.0	17.0	17.0	15.0	14.0	14.0	14.0	138.0
	7.6	17.0	17.0	17.0	17.0	17.0	17.0	16.0	16.0	16.0	150.0
	8.0	17.0	17.0	17.0	17.0	15.0	14.0	13.0	13.0	13.0	143.0
	8.4	14.0	14.0	14.0	14.0	14.0	14.0	13.0	13.0	13.0	124.0
	Total	147.	147.	147.	146.	144.	138.	131.	127.	127.	

very pale lemon at 5.2 to amber at 8.4. Differences in the color of the extracts were also observed in the preceding experiment, where the extracts in more concentrated phosphate were slightly darker, but the effect was much less than in the present experiment. The variation of color intensity with pH has been repeatedly observed in other subsequent experiments. It is of value since by comparing two extracts which have been placed under different experimental conditions (as in enzyme digestion) it is possible to make a rough estimate from the color as to the pH effect. This fact was made use of in later experiments. While speaking of the color of the extracts it might be well to mention, in passing, the effect of dialysis on the pigment in these extracts. In the many dialyses which have been employed in this study it has always been observed that the completely dialyzed dialyzates

always retained the same dark color as the undialyzed controls, i. e. the pigment is non-dialyzable. However a colorless color-base does pass readily through the membranes and if this colorless diffusate is boiled a rich brown color results, presumably from its oxidation.

Beside the color change due to pH there is another pH effect on the extracts which should be mentioned, namely, the tendency for more alkaline extracts to become cloudy or opalescent. Silberschmidt recognized this fact (l. c. p. 146: "das Filtrat wurde leicht trüb, vermutlich, da es etwas alkalisch war"). This effect was not noted below the point of neutrality, but it has been repeatedly observed in alkaline extracts. It is easily eliminated by making the extracts neutral or slightly acid, which results in a re-solution of the solid suspension.

A second experiment on the effect of pH is incorporated into an experiment on heat in the following section (page 130). The result of this experiment was to demonstrate that the reaction does not take place at pH 1.4-1.5 or at pH 8.8. This failure in reactivity may be due to one of two possibilities: either (a) the reactive substances are present in extracts which have been so titrated but are unable to react because of the excessive alkalinity or acidity of the solutions, or (b) the process of titration has resulted in a precipitation of the active ingredients, the reactivity of which is irrevocably lost if the coagulum is removed. The answer as to which of these two possibilities is the correct explanation is seen in the following experiment.

TABLE III.

EFFECT OF REMOVAL OF ALKALINE COAGULUM ON PRECIPITIN REACTION IN PRUNUS-PLATANUS-ROBINIA.

		Platanus		Robinia	
		Normal:	Alkaline supernatant:	Normal:	Alkaline supernatant:
Prunus	Normal:	4	4	3	3
	Alkaline supernatant:	4	4	3	3

Normal extracts of *Prunus*, *Platanus*, and *Robinia* were prepared (in distilled water). Each was divided into two fractions, one fraction being titrated to pH 9.0 with KOH. The abundant precipitate was then centrifuged away, and the supernatant fluid titrated with HCl back to approximately the normal pH. The extracts were then tested as is shown in Table III.

A study of this table reveals that the alkaline coagulum does not contain the reactive substances but that the supernatant fluid from

such coagulation does contain the reactive principles in undiluted strength, and that, by comparison with Table V, the reaction in the latter table is inhibited not by absence of reactive substances but by an excessively high pH, the reaction being fully restored when the pH is again brought to normal.

C. Effect of Heating on the Reactions

Having now determined the effects of salt concentration and pH upon the reactions, the next important step was to determine the effect of heat upon the extracts, for a sharp decline in reactivity of extracts which have been heated to temperatures of 60°-80°C. would yield strong evidence as to the nature of the reaction. Several experiments in heating the extracts have been performed, all yielding comparable results. As a preliminary word it should be mentioned that serologically active protein is usually coagulated at about 60°C., sera heated above this point and subsequently cleared having lost their specific reactive power. However, a somewhat different display of behavior might be anticipated with the plant extracts since according to Osborne (19, p. 65) "the seed proteins differ in a marked degree from the animal proteins, for most of them are very incompletely coagulated by heating their solutions even to boiling, and many of them are not coagulated at all under these conditions." Neutral and alkaline protein solutions are heat-coagulable only with difficulty or not at all, so that in the experiments reported below the extracts were all prepared under such conditions as to give them a slightly acid reaction.

In the first heat experiment performed, extracts of *Robinia* and *Prunus* were employed. A quantity of normal extract was prepared for each of the species, the solvent used being a Cohn's phosphate buffer of pH 6.0 and salt concentration of .12M (6). The two extracts were then each divided into 10 samples. One sample of each was untreated, the other 9 samples were each heated in water baths for ½ hour at varying temperatures from 30°C. to 100°C., so as to afford a complete series for *Robinia* and for *Prunus*. All the extracts were then clarified by centrifuging where necessary, and tested in all possible combinations. Clarification was unnecessary throughout in *Prunus* as the very slight opalescence which developed at the highest temperatures was so slight as not to interfere with reading the reactions. On the other hand, the *Robinia* extracts threw out a very dense and flocculent precipitate which began at 60° and became intense at 70°-100°C. The removal of these precipitates, however, as will be seen below, had little effect on the reaction. As controls for this experiment the tests of the

treated extracts against the normal extracts and then against one another suffice to eliminate possibilities of error due to non-experimental variables. The results of this first experiment are given in Table IV.

TABLE IV.

EFFECT OF HEAT UPON THE PRUNUS-ROBINIA PRECIPITIN REACTION.

		Robinia.....Heated 1/2 hour at temperature:									
		None	30°	40°	50°	60°	70°	80°	90°	100°	Total
	None	17.0	17.0	17.0	17.0	17.0	12.0	11.0	10.0	7.0	125.
	30°	17.0	17.0	17.0	17.0	17.0	12.0	11.0	10.0	7.0	125.
Prunus...	40°	17.0	17.0	17.0	17.0	17.0	12.0	11.0	10.0	7.0	125.
Heated 1/2 hour at:	50°	17.0	17.0	17.0	17.0	17.0	12.0	11.0	10.0	7.0	125.
	60°	12.0	12.0	12.0	11.0	10.0	7.5	6.5	5.5	6.5	83.
	70°	12.0	12.0	12.0	9.0	8.0	7.5	5.5	5.5	6.5	78.
	80°	11.0	12.0	10.0	8.0	8.0	7.5	5.5	5.5	5.5	73.
	90°	10.0	10.0	10.0	7.0	6.0	5.5	5.0	5.5	3.0	62.
	100°	9.0	9.0	9.0	7.0	6.0	5.5	4.0	5.5	3.0	58.
	Total	122.	121.	121.	110.	106.	81.5	70.5	67.5	52.5	

As will be seen from a study of the data here presented there is a decline in the reactivity of both extracts on being heated above 60°C. At first glance this would seem to indicate the direct effect of a protein coagulation. Indeed, such was the conclusion of the writers on first viewing the results. But subsequent experiments have shown this conclusion to be erroneous. The decrease observed at 60° is, to be sure, probably an effect of the heat coagulation, but an indirect effect, the coagulum of protein having by physical action withdrawn from solution, in coagulation, some of the other, non-protein substances present in the extracts.

As a further test of the effect of heating it was resolved to heat the extracts more thoroughly in an endeavor completely to eliminate the reaction. Accordingly normal extracts were prepared of *Prunus* and *Robinia* in distilled water. Each extract was divided into three samples, one sample being reserved as a control, the other two being titrated with HCl to pH 1.4 and with NH_4OH to pH 8.8 respectively. The unaltered extract of *Prunus* had a pH of 4.3, that of *Robinia* 4.8. Each sample was again divided into two, one fraction being heated, the other

being retained as a control. Heating was accomplished by autoclaving with flowing steam at a pressure of 2 lbs. for three hours. The extracts were then cleared by centrifuging and tested as in Table V.

In all cases there were coagulations due to heating as well as coagulations due to acidification and alkalinization. These were more con-

TABLE V.

EFFECT OF LONG CONTINUED HEATING ON THE PRUNUS-ROBINIA PRECIPITIN REACTION.

		Prunus....Unheated:			Prunus.....Heated:		
		pH 1.4	pH 4.3	pH 8.8	pH 1.4	pH 4.3	pH 8.8
Robinia (Unheated)	pH 1.5	0			0		
	pH 4.8		17			17	
	pH 8.8			0			0
Robinia (Heated)	pH 1.5	0			0		
	pH 4.8		17			17	
	pH 8.8			0			0

spicuous in *Robinia* than in *Prunus*. In view of this fact, the results of Table V are most interesting. There was no perceptible influence on the reaction due to this prolonged heating. All the reactions were repeated in parallel several times and viewed by two observers to confirm this point. It is thus manifest that the coagulum due to heating does not contain the reactive substances in these extracts and that accordingly the reaction is either due to a non-protein or to a protein highly resistant to heat. The effect of pH is here also worthy of comment. In the extracts which had been titrated there was a complete loss of reactivity even in the unheated controls. The statements made in the preceding section must accordingly be amended to include the conception that alteration of pH to very high or very low values completely eliminates the reaction, which view is compatible with the other findings reported. It need hardly be emphasized, however, that such pH values as 1.4 and 8.8 never have been observed to occur in extracts prepared in the ordinary fashion.

The effect of pH on the color of the extracts was again noted in this experiment, that of both extracts varying from light amber at 1.4 to dark mahogany at 8.8.

As further controls for this experiment the *Robinia* extracts at vari-

ous pH values, both heated and unheated were tested *inter se* with completely negative results. The same was true of the *Prunus* extracts. This completely eliminated any artefact due to the experimental procedure. Furthermore, reactions between any two extracts of different pH were not included because of the artefact reaction introduced through pH coagulation.

D. Effect of Dilution on the Reactions

One of the most striking effects of the precipitin reaction in animal serology is the zone effect, whereby an excess of reactive substance may inhibit the reactive display, where there is an optimum concentration of reactive principles, which optimum if passed in either direction, results in a decrease of reactivity. The question arises whether such a zone phenomenon is characteristic of the reactions being studied in this paper. The answer to this question will at once afford a significant

TABLE VI.

EFFECT OF EXTRACT DILUTION ON THE PRUNUS-PLATANUS PRECIPITIN REACTION.

		Platanus extract...Normality:													
		10	5	2	1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	Total
Prunus extract... Normality	10	29.0	27.0	24.0	20.0	15.0	14.0	12.0	10.0	7.0	4.0	1.5	1.0	0.0	164.5
	5	27.0	25.0	20.0	17.0	16.0	12.0	11.0	9.0	5.5	1.0	0.0	0.0	0.0	143.5
	2	24.0	21.0	16.0	15.0	9.0	8.0	7.0	5.5	4.0	0.0	0.0	0.0	0.0	109.5
	1	18.0	17.0	15.0	14.0	8.5	6.0	5.5	3.5	3.0	0.0	0.0	0.0	0.0	90.5
	1/2	13.0	12.0	12.0	11.0	8.0	6.0	4.5	3.0	2.5	0.0	0.0	0.0	0.0	73.0
	1/4	12.0	12.0	11.0	9.0	7.0	5.5	3.5	3.0	2.5	0.0	0.0	0.0	0.0	65.5
	1/8	12.0	12.0	11.0	7.0	5.0	4.5	2.5	0.0	0.0	0.0	0.0	0.0	0.0	54.0
	1/16	11.0	11.0	10.0	5.5	5.0	4.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0	49.0
	1/32	7.0	6.0	4.0	3.0	1.5	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	23.0
	1/64	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0
	1/128	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1/256	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1/512	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Total:	156.	145.	123.	101.	75.0	61.5	48.5	34.0	24.5	5.0	1.5	1.0	0.0	

comparison between the plant precipitations and those of serology and at the same time instruct as to the desirability of controlling this possible variable in subsequent work. Accordingly the following experiment was devised.

Normal extracts of *Platanus* and *Prunus* were prepared as usual.

Each extract was then boiled down to 1/10 its volume (reference to the work in heat effects assuring one that this would not seriously interfere with the reactivity of the extracts). They were then centrifuged. Using these 10 normal supernatants as bases, progressive series of dilutions were made. Then each grade of *Prunus* was tested against the various grades of *Platanus* according to the scheme of Table VI.

The readings were made by the method of summation defined above, and the totals plotted against the normalities of the extracts as in Figure I.

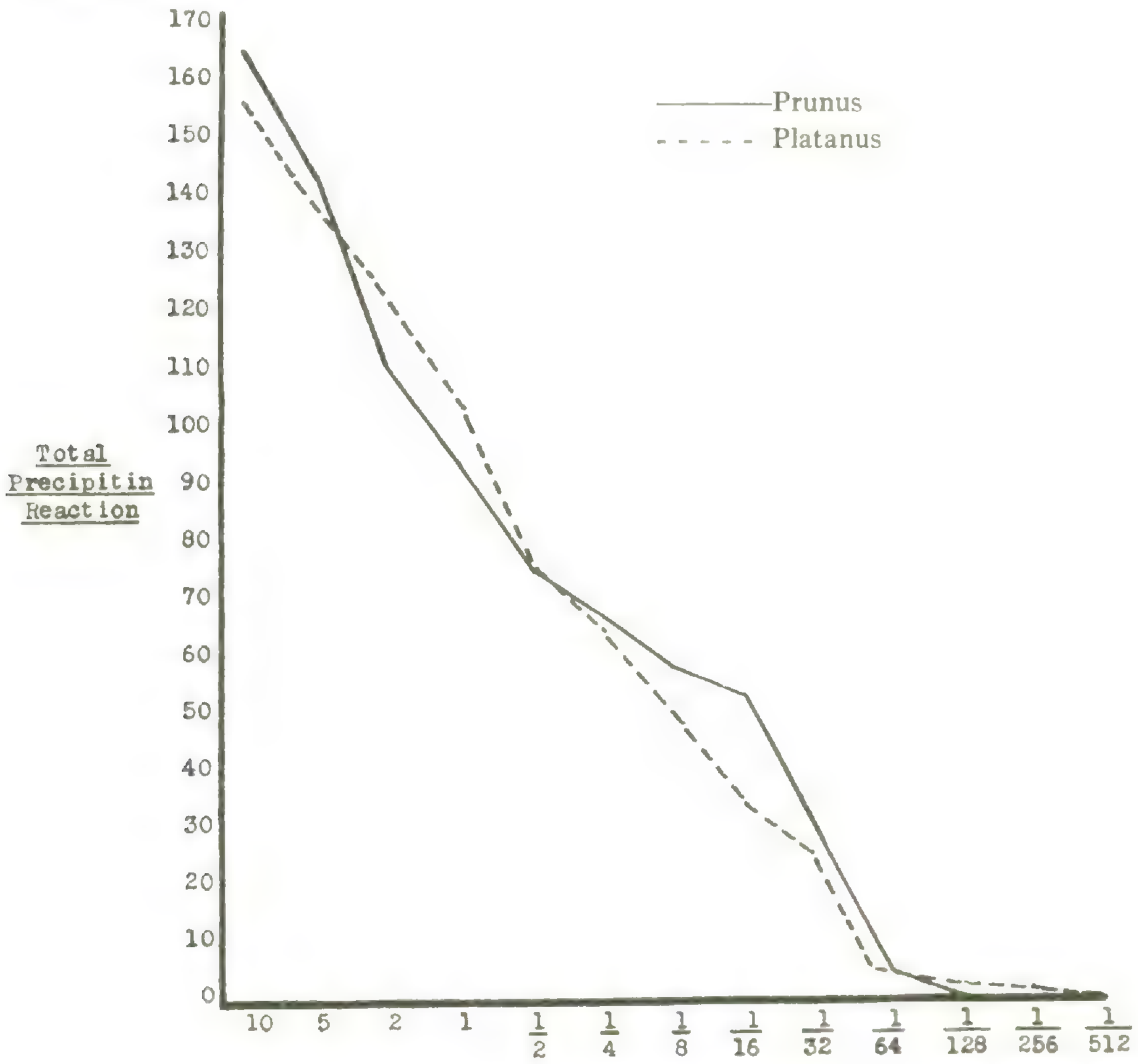


FIGURE I. VARIATION IN THE PRUNUS-PLATANUS PRECIPITIN REACTION WITH PROGRESSIVE DILUTION.

It is at once apparent from a study of Table VI and Figure 1 that dilution of the extracts causes only a regular dilution of reactivity. There is not the slightest indication of a zone effect. In this respect, then, the plant reaction under consideration exhibits a marked differ-

ence from precipitin reactions in animals, and the evidence against an homologous reaction with that of mammalian blood is accordingly strengthened. The relative regularity of the curves also affords a measure of the relative accuracy of the method of reading, since there is no appreciable deviation at any point of the curves.

E. Effect of Dialysis on the Reactions

The next, and probably the most important step in determining the nature of the reactions under consideration is the dialysis of the extracts with subsequent testing. If the reactive substances are completely or nearly completely held back by protein-impermeable dialyzing membranes, then there is strong but not absolute proof that the reaction is due to proteins or to radicals bound to proteins. If, on the other hand, the reactive substances easily pass through membranes which are independently proved to be impermeable to proteins, then the evidence is conclusive that the reactive substances are non-protein in nature. Accordingly great stress is here laid on the dialysis experiments reported.

The technique of dialysis varied at the start until a technique was found which proved to be wholly satisfactory. For membranes, collodion and cellophane were first employed but they proved to have numerous disadvantages in comparison to the parchment which succeeded them. The collodion membranes, in addition to the labor of making them, proved much more difficult to handle and there was the greater danger of their rupturing or being imperfectly constructed, while the cellophane (commercial: Dupont Corp. #300, not waterproof) proved to be so frequently imperfect that not more than one out of five sheets was satisfactory. Moreover the cellophane when wet is very easily broken and is handled only with difficulty. A high grade of parchment, first in the form of diffusion cells, later in sheets, proved entirely satisfactory. Although every sheet was tested in these experiments, very few were found to require rejection.

As regards apparatus, the first designed was in the form of the customary shallow tray with a periodic water change, in which the liquid to be dialyzed was placed in diffusion shells. This was efficient but very slow in contrast to the other apparatus described below. Using the diffusion shells two weeks were necessary for a satisfactory dialysis (at 2°C.), and since the time was so extended there was danger of errors due to this long standing.

A dialysis device employed by Dr. F. D. Hagar at the Massachusetts Antitoxin and Vaccine Laboratory was studied and then modified to meet the conditions of this study. The apparatus is constructed according to the plan of Figure II.

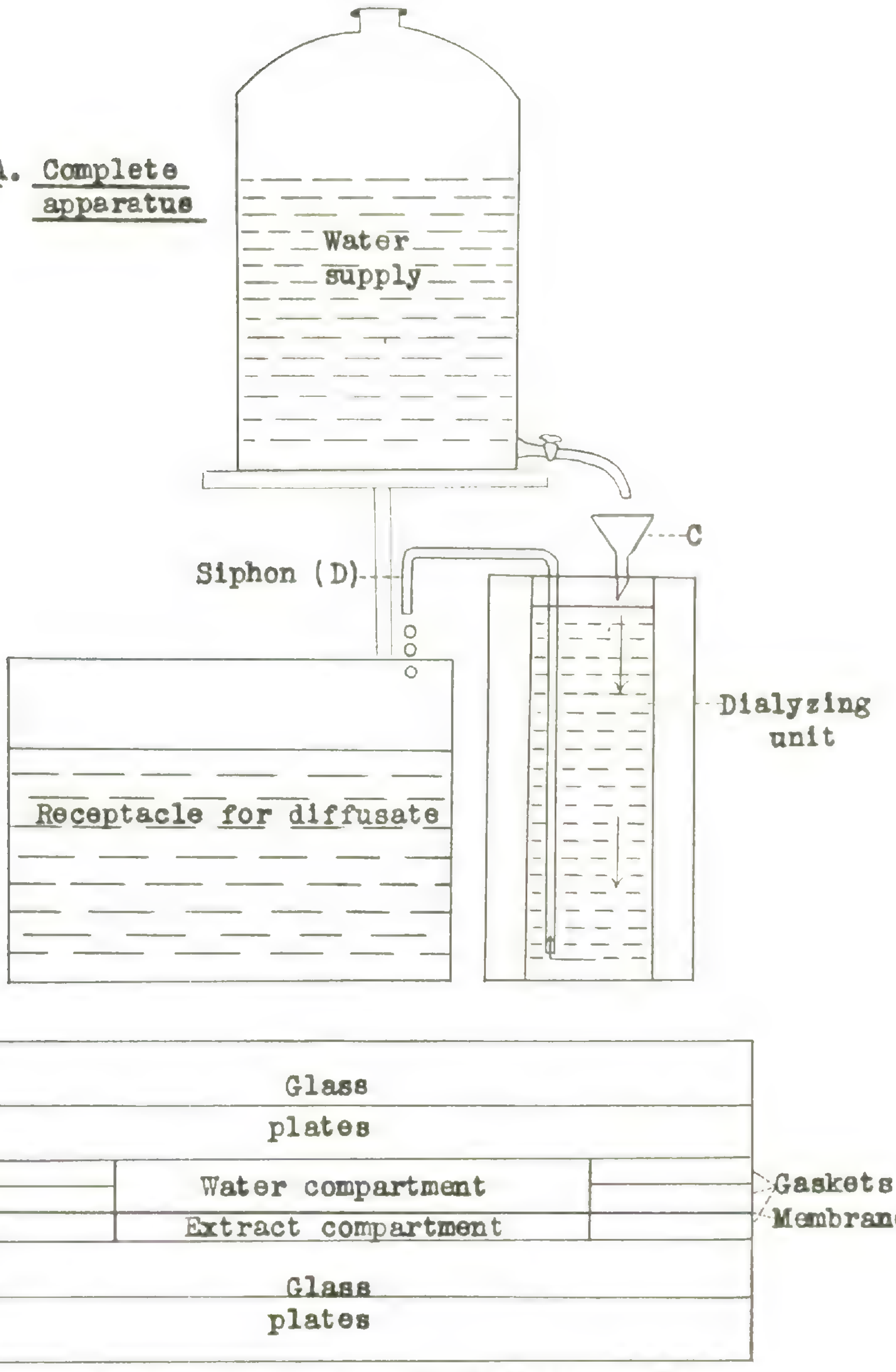


FIGURE II. APPARATUS FOR DIALYSIS OF EXTRACTS.

The dialyzing membrane, cut to a size of 8″ x 4″ (or preferably narrower) is fixed between two U-shaped rubber gaskets of the same size, one gasket being twice the thickness of the other. An automobile inner tube of heavy, smooth rubber may be used for the construction of the gaskets. These gaskets are in turn clamped between two heavy glass plates. The glass must be rather thick (¾″, or we have used two plates on each side of ⅜″-½″ thickness each). On the side of the membrane separated from the glass by the thinner gasket is placed the extract to be dialyzed. The other side of the membrane is in contact with a moving stream of water, entering at the funnel C and leaving by the siphon D. Such an apparatus affords a high degree of efficiency and has the advantages of affording a continuous flow, yet of being small enough to be placed in an ordinary sized refrigerator. About 25 cc. of extract may be dialyzed at a time, although by constructing the apparatus on a smaller scale this could be reduced to 5 cc. if desirable. The water flow is at about the rate of two litres per day, and dialysis for 1-2 days has given perfectly satisfactory results. At first the dialyses were carried out at 2°C., but later tests showed that a dialysis for 24-48 hours at room temperature did not seriously interfere with the reactivity or clearness of the liquids, and accordingly most of the experiments reported below were performed at room temperature.

Using such an apparatus the diffusate may be saved and tested in addition to the dialyzate if such proves desirable. The experiments on the *Prunus-Platanus-Ribes-Robinia* reactions soon showed that the reactive principles passed rapidly through the membranes, and accordingly the diffusates were studied. These were evaporated by boiling in small fractions and thus reduced to the original volume of the dialyzed extracts. Control tests were employed in which whole extracts were diluted to 3-4 litres and then boiled down. Since there was no reduction in reactivity in such controls, it was assumed that the technique of boiling down the diffusate was justified.

The efficiency of the membranes was tested in several ways. In the first place, a set of preliminary tests were made with the parchment to determine their permeability. These experiments are tabulated in Table VII. The tests for the presence or absence of substances in dialyzate and diffusate included the Millon and xanthoproteic tests for protein, the Ninhydrin test for protein cleavage products, the iodine test for starch, the Fehling test for sugar, and the silver nitrate test for chloride. These tests are all highly sensitive. The Millon test reveals the presence of one part of peptone in 3000 parts of water, the Ninhydrin test one part of peptone in 50,000 parts of water (water-clear),

and the Fehling test is sensitive to at least one part of glucose in 2000 parts of water (these sensitivities being independently determined for this study). The sensitivity of the $AgNO_3$ test is also very high, that of iodine less so.

TABLE VII.

EXPERIMENTS IN DIALYSIS: TESTING OF PARCHMENT MEMBRANES.

Exp.	Substance dialyzed:	Nature of extract:	Dialysis tests for: Protein: Millon	Protein: Xanthoproteic	Protein: Ninhydrin	Starch: Iodine	Sugar: Fehling	Cl: $AgNO_3$
A	Peptone + glucose	Normal	5		5		5	
"	"	Dialyzate	5		5		5	
"	"	Diffusate	0		1		3	
B	Ovalbumin + starch + glucose + NaCl.	Normal	4	4		3	5	5
"	"	Dialyzate	4	4		3	4	4
"	"	Diffusate	0	0		1	3	3
C	"	Normal	4	4		3	5	5
"	"	Dialyzate	4	4		3	4	4
"	"	Diffusate	0	0		1	3	3

In Experiment A the solution to be dialyzed contained peptone (saturation) and glucose (.2%) in water. Dialysis lasted for 18 hours and there was no continuous flow of water in the apparatus, which contained 30 cc. of dialyzate and 40 cc. of diffusate. At the end of this experiment the tests showed that the peptone had passed through only in very small amount, while the glucose had passed through freely.

In Experiment B the solution to be dialyzed consisted of powdered egg albumen (.5%), soluble starch (.2%), d-glucose (.25%), and NaCl (about .2%). The clear filtered solution was dialyzed for 24 hours with no flow in the apparatus. The tests at the end of this time showed that no albumen was demonstrable in the diffusate, while it was unaltered in strength in the dialyzate, that the sugar and salt had passed through freely, and that starch had passed through but not so freely as the sugar and salt.

Experiment C was a repetition in all respects of Experiment B, and it will be seen that the results were exactly the same as in the preceding experiment.

Thus these three membranes, chosen at random, showed a porosity of the exact type required for this work, and offered confidence that the use of such membranes would be of value in the succeeding experiments.

Beside these preliminary tests, each dialysis by itself was tested for degree of dialysis by employing on controls, dialyzates, and diffusates tests for protein, carbohydrates, and salts. The Millon, Fehling, $AgNO_3$,

and oxalate tests were extensively used. The xanthoproteic and iodine tests were also used but less frequently, because the former is of little value in strongly colored extracts, while the latter is less sensitive than the other tests. It is to be emphasized that there is no intention that the presence or absence of Millon-testable protein directly indicates the presence or absence of (hypothetical) precipitin-active protein. Rather these chemical tests are to be considered tests of the degree of dialysis and nothing more. Thus if the diffusate of any given experiment shows no Millon protein, then it is a natural assumption that all protein is being reasonably well retained, while if the diffusate gives a strong $AgNO_3$ or Fehling test, this is indicative that all crystalloids of the same approximate molecular size are passing freely through the membrane.

In each experiment a control tube of the same extract as was being dialyzed was placed under corresponding conditions, at room temperature or at 2°C. respectively throughout the experiment, and then used as "Normal" in the dialysis tests. The protein, salt, and precipitin tests were all performed in the customary manner with the usual controls. All the dialyses lasted from 1-2 days, and all were performed using distilled water as the fluid outside the membrane. The results of the dialyses with *Prunus*, *Platanus*, and *Robinia* are here introduced in tabular form (Tables VIII-XI) since space does not permit the introduction of an intimate discussion of each experiment.

Considering first the reactions of *Platanus acerifolia* (Table VIII) the control extracts in every case contained strong protein, chloride, and sugar as is shown by the tests. The controls were all highly and equally reactive with *Prunus* and completely negative with *Robinia*, in conformity with the earlier experiments. In dialysis, except in one experiment (Exp. 9) the Millon protein was completely held back by the membranes, while the salt passed freely through. It is thus highly significant that the diffusates in every case offered strong reactions with the *Prunus*, while the dialyzates, although not negative in but one case, exhibited only very weak reactions. The membrane in Experiment 7 appeared to have been far more retentive than any of the others, as is shown by all the tests, and accordingly the results in this experiment are of less significance than the others. Thus it is evident that there is a definite and strong correlation between the diffusable substances through protein-impermeable membranes and the precipitin potency. The fractionation is not perfect, but in such experiments as these the significance depends not upon a single test but upon the trend exhibited by all. That the positive reactions of the diffusates were not artefacts

TABLE VIII.

EXPERIMENTS IN DIALYSIS: DIALYSES OF PLATANUS.

Nature of extract:	Exp. #:	Dialysis tests for: Protein	Chloride	Sugar	Precipitin tests against normal: Extract:	Test:	Extract:	Test:
Normal	5	4	4	4	Prunus	4	Robinia	0
"	6	4	4	4	"	4	"	0
"	7	4	4	4	"	4	"	0
"	8	4	4	4	"	4	"	0
"	9	4	4	4	"	4	"	0
"	10	4	4	4	"	4	"	0
Dialyzate	5	3	1	0	"	1	"	0
"	6	3	1	0	"	1	"	0
"	7	3	3	0	"	3	"	0
"	8	3	1	0	"	1	"	0
"	9	4	1	0	"	0	"	0
"	10	4	2	1	"	1	"	0
Diffusate	5	0	4	4	"	4	"	0
"	6	0	4	4	"	4	"	0
"	7	0	4	4	"	4	"	0
"	8	0	4	4	"	4	"	0
"	9	1	4	4	"	4	"	0
"	10	0	4	4	"	4	"	0

TABLE IX.

EXPERIMENTS IN DIALYSIS: DIALYSES OF ROBINIA.

Nature of Extract:	Exp. #:	Dialysis tests: Protein	Chloride	Precipitin tests against normal; Extract:	Test:	Extract:	Test:
Normal	19	4	4	Prunus	4	Platanus	0
"	21	4	4	"	4	"	0
"	22	4	4	"	4	"	0
"	23	4	4	"	4	"	0
"	24	4	4	"	4	"	0
"	25	4	4	"	4	"	0
"	26	4	4	"	4	"	0
Dialyzate	19	3	1	"	1	"	0
"	21	4	2	"	2	"	0
"	22	2	1	"	0	"	0
"	23	4	1	"	1	"	0
"	24	3	1	"	1	"	0
"	25	4	2	"	2	"	0
"	26	4	2	"	2	"	0
Diffusate	19	2	4	"	4	"	0
"	21	1	4	"	3	"	0
"	22	2	4	"	3	"	0
"	23	2	4	"	4	"	0
"	24	2	4	"	4	"	0
"	25	1	4	"	3	"	0

TABLE X.

EXPERIMENTS IN DIALYSIS: DIALYSES OF PRUNUS.

Nature of extract:	Exp. #:	Dialysis tests: Protein	Chloride	Sugar	Precipitin tests against normal: Extract:	Test:	Extract:	Test:
Normal	1	4	4	4	Platanus	4	Robinia	4
"	2	4	4	4	"	4	"	4
"	3	4	4	4	"	4	"	4
"	4	4	4	4	"	4	"	4
"	11	4	4	4	"	4	"	?
"	12	4	4	4	"	4	"	?
"	13	3	4	4	"	4	"	2
"	15	4	4	4	"	4	"	4
Dialyzate	1	3	1	0	"	0	"	0
"	2	3	1	1	"	0	"	0
"	3	3	1	0	"	0	"	0
"	4	3	1	0	"	0	"	0
"	11	4	2	0	"	0	"	?
"	12	4	2	0	"	0	"	?
"	13	2	1	0	"	0	"	0
"	15	3	2	0	"	0	"	0
Diffusate	1	0	4	?	"	4	"	4
"	2	0	4	4	"	4	"	4
"	13	1	3	3	"	2	"	2
"	15	2	4	4	"	3	"	3

TABLE XI.

EXPERIMENTS IN DIALYSIS: TESTS OF FRACTION AGAINST FRACTION (PRUNUS-PLATANUS)

Prunus: Exp. #	Nature of extract:	Dialysis tests: Protein	Chloride	Sugar	Platanus: Exp. #	Nature of extract:	Dialysis tests: Protein	Chloride	Sugar	Precipitin reaction
11	Dialyzate	4	2	0	9	Dialyzate	4	1	0	0
12	"	4	2	0	9	"	4	1	0	0
15	"	3	2	0	9	"	4	1	0	0
1	Diffusate	0	4	4	5	"	3	1	0	0
1	"	0	4	4	6	"	3	1	0	0
1	"	0	4	4	7	"	3	3	0	0
1	"	0	4	4	8	"	3	1	0	0
15	"	2	4	4	9	"	4	1	0	0
1	Dialyzate	3	1	0	10	Diffusate	0	4	4	0
2	"	3	1	1	10	"	0	4	4	0
3	"	3	1	0	10	"	0	4	4	0
4	"	3	1	0	10	"	0	4	4	0
11	"	4	2	0	9	"	1	4	4	0
12	"	4	2	0	9	"	1	4	4	0
1	Diffusate	0	4	?	10	"	0	4	4	4
2	"	0	4	4	10	"	0	4	4	4
1	"	0	4	4	5	"	0	4	4	4
1	"	0	4	4	6	"	0	4	4	4
1	"	0	4	4	7	"	0	4	4	4
1	"	0	4	4	8	"	0	4	4	4

is demonstrated by the fact that they were absent when tested against *Robinia* and also when tested against one another (additional control tests not shown in the table).

In Table IX the results with *Robinia fertilis* are entirely comparable. Here the retention of protein is not as complete as in *Platanus* (though it must be remembered that the Millon test is positive not only to formed protein but also to certain of its cleavage products, e.g. peptone), but the dialysis results in a fractionation in which one fraction, the diffusate, contains the bulk of the crystalloids, and the other fraction, the dialyzate, the bulk of the protein. The fraction containing most of the crystalloids contains most of the precipitin-reactive substances, and the fraction containing most of the protein shows only weak precipitin reactions.

The results with *Prunus Armeniaca* (Table X) are most striking of all. Here the fractionation resulted in diffusates which were almost or entirely protein-free according to the Millon test but which contained the great bulk of the crystalloids, while the dialyzates showed little protein loss and little crystalloid retention. Subsequent tests have shown that the chloride which seems necessarily retained with the protein is probably bound to the protein and is hence non-dialyzable, since the equally delicate oxalate test in later experiments showed such dialyzates to be perfectly calcium-free. The high-protein, low-crystalloid dialyzates were perfectly negative to active extracts of *Platanus* and *Robinia,* while the high-crystalloid, protein-negative diffusates were richly endowed with precipitin potency.

The next table, Table XI, shows the tests of fraction against fraction. These tests were performed to obtain confirmatory data to Tables VIII-X and also to answer a question that arose from a consideration of the data in these preceding tables. It is known that in animal precipitin reactions the presence of a small amount of salt is necessary in order that the reactions take place. In testing a dialyzate against a diffusate or a normal extract one would expect that if the loss of reaction were due to a loss of salt, the second extract would supply sufficient electrolyte to permit the reaction to take place. Such does not occur (see Tables VIII-X as well as XI). On the other hand, if the reaction is due to protein in either extract it would be highly improbable that the testing together of two Millon-negative diffusates could produce a reaction equivalent in strength to the original reaction of the normal extracts. Granting that a trace of protein, non-testable by the Millon test, may have escaped through the membranes, could this give a positive reaction *equal in strength to the controls?* One could hardly

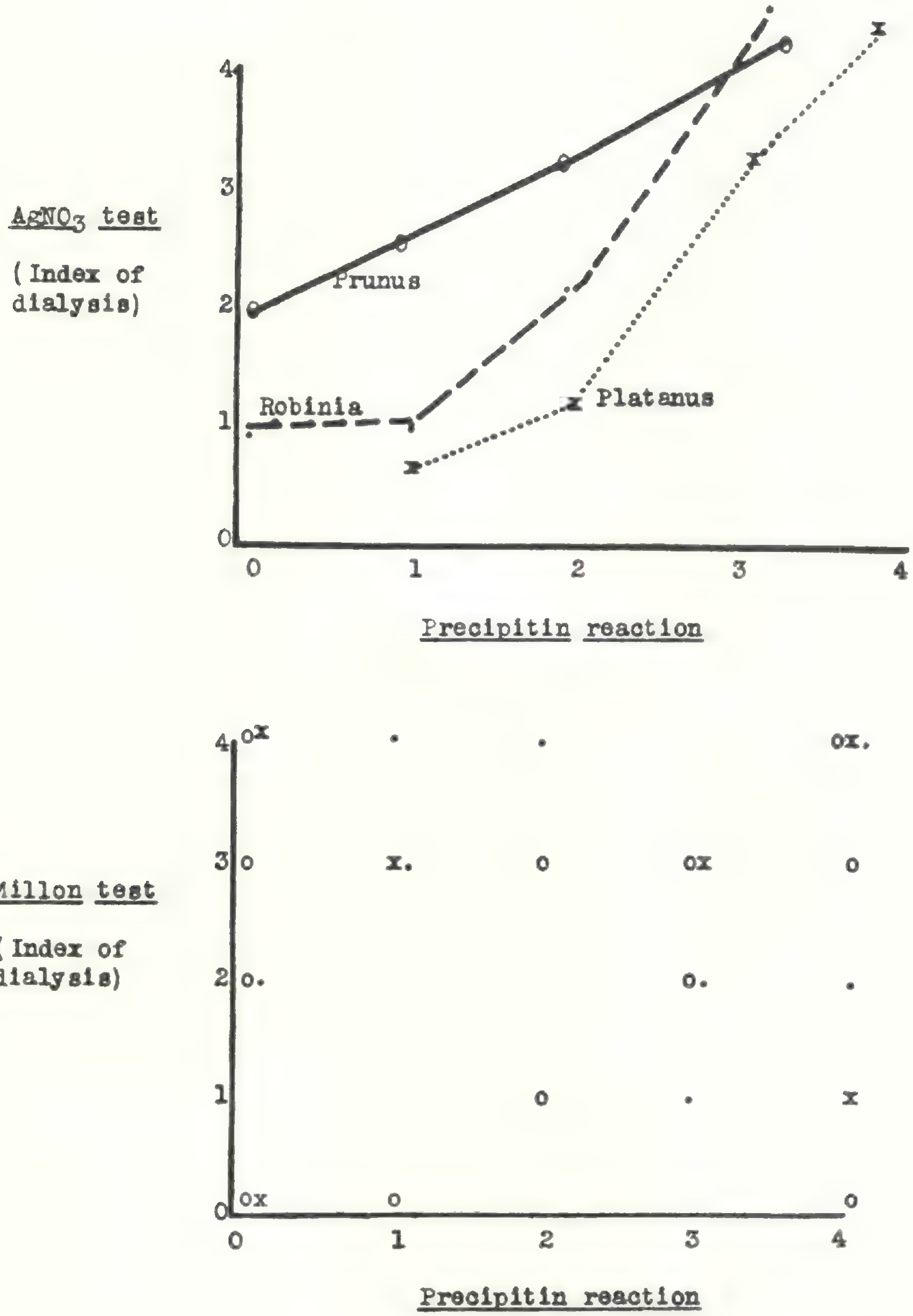


FIGURE III. EFFECT OF DIALYSIS ON THE PRUNUS-ROBINIA-PLATANUS PRECIPITIN REACTION.

KEY: o = Prunus; . = Robinia; x = Platanus. O = no test; 1 = very weak test; 2 = weak test; 3 = moderate test; 4 = strong test.

cling to such a remote possibility in the present case, for the dialyzates are in no case positive to one another nor to the diffusates, while the diffusates are in every case not only positive *inter se*, but positive to a strength *equivalent to that of the normal control reaction*. The results of all these experiments are summarized in the graphs of Figure III which illustrate strikingly that the reactive substances bear a direct and intimate relationship to the dialyzable crystalloids and none whatever to the non-dialyzable proteins.

The experiments in dialysis thus prove definitely that whatever the nature of the *Platanus-Prunus-Robinia* reactions, the substances responsible for them pass freely through membranes highly impermeable to protein. This applied equally to the reactive principles of *Prunus*, *Platanus*, and *Robinia*. It is thus inconceivable that the precipitin reactions among these species could be due to protein unless one clings to the very doubtful hypothesis that a very small fraction of protein may give as strong a precipitin test as a quantity of protein 10 or 100 times as great, provided that electrolytes be present in sufficient quantity.

F. Effect of Various Solvents on the Reactions

Several types of experiments have been undertaken to demonstrate what solvents will dissolve the reactive principles of *Platanus*, *Prunus*, *Robinia*, and *Ribes*, as well as what solvents will denature the reactive principles. The solvents employed have been alcohol, ether, benzol, chloroform, and carbon tetrachloride. The purposes of the experiments were various, first, to investigate the possibility of improving the technique by an extraction process which will eliminate many heterologous substances from the extracts, second, to shed light upon the chemical nature of the active principles by determination of their various solubilities, third, to observe the effect of denaturing, particularly with regard to protein, upon the reaction, and fourth, to determine whether the techniques used by Silberschmidt in pre-extraction and solution exert any significant effect on the reactions at present being considered.

The first experiment performed upon this topic was one designed to determine whether the reactive substances of *Platanus* and *Prunus* are soluble in alcohol of various strengths. Samples of leaf powder of these species were extracted in the customary manner but using as solvents water, 20%, 40%, 60%, 80%, and absolute alcohol. These extracts were filtered, and then tested for their content in protein, sugar, and chloride, and for their precipitin potency. It was not possible to test all the possible combinations since the addition of a strong alcohol to a

water solution or one in weak alcohol resulted in an artefact coagulation effect. The results of this experiment are given in Table XII.

It is seen that there is a steady fall in precipitin potency as the strength of alcohol is increased until there is no reaction in alcohol of 60% or stronger. A comparison with the chemical tests shows that

TABLE XII.

DIRECT EFFECT OF ALCOHOL UPON THE PRUNUS-PLATANUS PRECIPITIN REACTION.

		Prunus: Extracted in alc. of strength:					
		100%	80%	60%	40%	20%	0%
	Protein:	2	2	3	4	1	3
	Sugar:	?	3	3	3	4	4
	Chloride:	0	t	1	2	3	4

Extr.	Prot.	Sug.	Cl.					
100%	t	?	0	0.0				
80%	2	3	t		0.0			
60%	3	3	1			0.0		
40%	4	3	2				11.0	
20%	4	4	3					13.0
0%	3	4	4					17.0

Platanus

there was no correlation between precipitin test and sugar (Fehling) or between precipitin reaction and protein, while there was an excellent correlation between the precipitin reaction and salt. This is of significance only by analogy, but the analogy is striking. It so happened in this experiment that the 20% alcohol solution of *Prunus* showed much weaker protein than either of the adjacent grades, but this is by no means reflected in the precipitin tests. The same reasoning applies to the water extract of *Platanus*. Thus this experiment informs us that the precipitin reactive substances are progressively less soluble in the increasing strengths of alcohol, and that the same is true of the chloride present in the extracts, although it is equally true of neither the protein nor the sugar.

An important question arises from this experiment, namely: Why were the extracts in strong alcohol non-reactive? Was it because the active substances are insoluble in alcohol or was it because they are

denatured by the stronger alcohol? This question was answered by the following experiment.

Leaf powders of *Platanus*, *Prunus*, and *Robinia* were extracted for 24 hours at 2°C. each with water, 60% alcohol, and 95% alcohol in three lots. The water extracts were then filtered and put on ice. The alcohol extracts were likewise filtered and the filtrates put aside. The alcoholic residues were dried and then extracted in water for 12 hours at 2°C. These last water extracts of the alcoholic residues were then filtered and all the extracts were tested in the manner shown in Table XIII.

TABLE XIII.

EFFECT OF ALCOHOL UPON THE PRUNUS-PLATANUS-ROBINIA PRECIPITIN REACTION.

			Prunus:			
				60% alcohol.	95% alcohol.	
			Water control	Alcohol extract	Water extract	Alcohol extract	Water extract
Water cont....4				4		4
	60% alc.	Alc. extr....		1		
		Water extr....4		3		4
	95% alc.	Alc. extr....			0	
		Water extr....4		4		4
Water cont....3				3		3
	60% alc.	Alc. extr....		1		
		Water extr....3		2		3
	95% alc.	Alc. extr....			0	
		Water extr....3		3		3

The results of this experiment are very striking. *Prunus*, *Platanus*, and *Robinia* all behaved similarly with respect to alcohol extraction. In no case was there evidence that 24 hours in alcohol had to any extent whatever weakened the reaction. Solution in 95% alcohol dissolved none of the reactive principles. Solution in 60% alcohol dissolved a small fraction of the reactive principles in all cases. One may say with assurance, therefore, that the reactive principles involved in these extracts are insoluble but not denatured, under these experimental conditions, by strong alcohol.

Proceeding next to a consideration of the same questions as were

raised regarding alcohol, but respecting lipoid solvents, the following experiment was performed. Dried tissues of *Prunus*, *Robinia*, and *Platanus* were thoroughly extracted with many changes of (a) ether, (b) chloroform, (c) benzol, and (d) carbon tetrachloride, in separate samples. The pulps were then dried and extracted in water in the customary way. Meanwhile other samples of each species were extracted in water without the lipoid pre-extraction. The extracts were then all tested for their precipitin potency. The scheme followed in testing may be illustrated in a single example (Table XIV).

TABLE XIV.

EFFECT OF LIPOID PRE-EXTRACTION ON PRUNUS-PLATANUS PRECIPITIN REACTION.

	Pre-extraction with ether: Strength of precipitin reaction after:						
Extracts tested together:	1 min.	5 min.	10 min.	20 min.	30 min.	40 min.	Total reac.:
Prunus control + Robinia control..	2	3	3	3	3	3	17
" treated + " control..	2	3	3	3	3	3	17
" control + " treated..	2	3	3	3	3	3	17
" treated + " treated..	2	3	3	3	3	3	17

It will be seen that the ether extraction removed no detectable quantity of the precipitin-reactive substances. Since space is limited the corresponding tables for chloroform, carbon tetrachloride, and benzol are omitted, but were they included they would simply be a duplication of Table XIV. In other words, long continued pre-extraction of these tissues with ether, chloroform, carbon tetrachloride, or benzol in no case resulted in the withdrawal of any appreciable amount of reactive substance. We may thus conclude that the reactive principles here are not of lipoid nature.

Further tests were employed with regard to benzol, in order to confirm the preceding experiment. Leaf powders of *Prunus*, *Platanus*, and *Robinia* were divided into two samples each. One sample was extracted in water as a control, a second sample was extracted for two hours at room temperature in commercial benzol. The benzol was filtered off, part being saved (A), the remainder being evaporated to dryness and re-dissolved in water (B). The benzol residue was dried and extracted in water (C). The various possible precipitin tests were then performed. The results of the tests showed that in every case the extract prepared from the benzol filtrate was perfectly negative whether the test was performed in benzol (A) or in water (B), while the reactions of the extracts prepared from the benzol residues (C) were equal in strength to those of the control extracts which had experienced no pre-extraction.

A last experiment to test the solubility of the precipitating principles in ether and benzol took the following form. Leaf powders of *Prunus*, *Platanus*, and *Robinia* were divided each into two samples. One sample was extracted directly in water. The other was first extracted in cold C. P. anhydrous ether, the extraction being continued as long as color was present in the filtrate (about 15 changes in 3 hours). These last samples were then divided as follows: 1 gm. of each when dry was placed in a tube with 10 cc. of water and extracted for 2 hrs. at room temperature (Table XV: *Ether controls*). The latter were then fil-

TABLE XV.

EFFECT OF BENZOL PRE-EXTRACTION ON PRUNUS-PLATANUS-ROBINIA PRECIPITIN REACTION.

		Precipitin reac. of:				
		Normal control	Ether control	Benzol filtrate; In benzol	Benzol filtrate; In water	Benz. res. In water
Experimental:	Experimental:					
Prunus	+ Platanus	3	3	0	0	3
Prunus	+ Robinia	3	3	0	0	3
Platanus	+ Robinia	0	0	0	0	0
Experimental:	Normal:					
Prunus	+ Platanus	3			0	3
Prunus	+ Robinia	3			0	3
Platanus	+ Prunus	3			0	3
Platanus	+ Robinia	0			0	0
Robinia	+ Prunus	3			0	3
Robinia	+ Platanus	0			0	0

tered. Of the ether residue 2.5 gm. of each species was next extracted in commercial benzol. Many changes were employed, extending through two days. The last change was with boiling benzol, the others with cold benzol. After each extraction (lasting about 1 hour), the benzol was decanted and filtered. The filtrate was evaporated in moderate heat. Previous to final evaporation the clear benzol filtrates were tested against one another (Table XV: *Benzol filtrate in benzol*). After complete evaporation the evaporated residue was re-dissolved in water, which was brought to boiling momentarily and then allowed to cool and extract over night. The following morning the water extract was cleared and tested (Table XV: *Benzol filtrate in water*). Meanwhile the benzol residue of powder was dried (with momentary boiling at the start), and extracted in water over night (Table XV: *Benzol residue*). In this, as in all the preceding and subsequent experiments the ratio of dried tissue to solvent was always 1:10, regardless of the solvent or amount of tissue. The results of these tests are seen in Table XV.

These last experiments thus confirm the earlier ones in demonstrating

that thorough extraction with lipoid solvents neither dissolves nor denatures the reactive substances.

As has been mentioned above, Silberschmidt has devoted much of his critique of technique (21) to the methods of pre-extraction and solution. As it was thought that Silberschmidt's results might have depended to considerable extent upon his methods of extraction, and as, further, it was desirable that the results of Kostoff and of Chester be reduceable to the same terms as Silberschmidt's, it was of much interest to determine the effect of Silberschmidt's techniques on our own material. Accordingly the following experiment was designed to include within one experiment all of the techniques described and used by Silberschmidt.

Homogeneous samples of dried leaves of *Prunus*, *Platanus*, and *Ribes* were extracted according to the following scheme:

Experimental tube number:	Pre-extraction:	Solution:
1	None	Water
2	None	Physiological saline
3	None	Water + solid NaCl to form phys. sol.
4	None	Phys. NaCl + MgO to neutrality
5	None	N/20 NaOH
6	95% alc. + 1% tartaric acid (5 hrs.)	Water
7	Ditto	Physiological NaCl
8	Ditto	Phys. NaCl + MgO to neutrality
9	95% alc. + tartaric acid 1% (5 hrs.); ether (5 hrs.); chloroform (5 hrs.); chloroform vapor (18 hrs.).	Water
10	Ditto	Water + solid NaCl to form phys. sol.
11	Ditto	Physiological NaCl
12	Ditto	Phys. NaCl + MgO to neutrality

All of solutions 6, 7, and 8 for any given species were made from the same pre-extracted tissue, and the same applies to 9, 10, 11, and 12 for each species. In pre-extracting there were three changes of each pre-extractant, the first change being momentary, the second lasting ½ hour, the third 1½ hrs. The pre-extracted tissues were then dried and extracted for 2 hours, each in its proper solvent. In decanting, in all the experiments in which this process was employed, it was cus-

tomary to decant repeatedly through the same filter paper, adding the residue in the paper to the leaf tissue being extracted. The various experimental extracts were then tested against normal extracts according to the plan in Table XVI.

TABLE XVI.

APPLICATION OF SILBERSCHMIDT'S METHODS OF PRE-EXTRACTION AND SOLUTION TO THE PRUNUS-PLATANUS-RIBES REACTION.

Extract number regardless of species	Exper. Platan. + Normal Prunus	Exper. Ribes + Normal Prunus	Exper. Prunus + Normal Platan.	Exper. Prunus + Normal Ribes	Exper. Prunus + Ca-free Platan.	Exper. Platan. + Ox-free Prunus	Exper. Ribes + Ox-free Prunus	Exper. Prunus + Ca-free Ribes
1	3	3	3	3	0	0	0	0
2	3	3	3	3	0	0	0	0
3	3	3	3	3	0	0	0	0
4	3	3	3	3	0	0	0	0
5	3	3	3	3	0	0	0	0
6	3	3	3	3	0	0	0	0
7	3	3	3	3	0	0	0	0
8	3	3	3	3	0	0	0	0
9	3	3	3	3	0	0	0	0
10	3	3	3	3	0	0	0	0
11	3	3	3	3	0	0	0	0
12	3	3	3	3	0	0	0	0

In this table "Normal" *Prunus*, *Platanus*, and *Ribes* refer to extracts #1 of these species respectively (aqueous extracts in which there had been no pre-extraction). A word of explanation must be inserted with regard to the last four columns of this table. At the time that this experiment was performed, the authors had already determined that the reaction in these species is due to the interaction of calcium ion in *Platanus* and *Ribes* with oxalate ion in *Prunus*. Therefore it was possible not only to determine the effect of the various extraction methods upon the calcium oxalate reaction, but it was also possible to eliminate the calcium oxalate reaction from consideration and observe whether these various techniques resulted in the demonstration of any additional reaction beyond that due to calcium oxalate. Accordingly, in the last four columns of Table XVI the "Normal" extracts are extracts in which

in the case of *Prunus* the oxalate present had been precipitated by the addition of a slight excess of $Ca(NO_3)_2$, and in the cases of *Platanus* and *Ribes* the calcium present had been precipitated by the addition of a slight excess of $K_2C_2O_4$.

Returning now to the experiment proper, one first observes that none of the various techniques of pre-extraction and solution made the slightest difference in the strength of reaction resulting, in any of the three species used. This demonstrates that there is no step in Silberschmidt's techniques which will eliminate the calcium oxalate reaction, and further that his techniques do not modify the calcium oxalate reaction, in these plants at least. The experiment also further confirms the earlier findings reported in this paper with regard to solubility of reactive substances in the various solvents employed. Lest the objection be raised that there might have been different reactions from the extracts obtained by the different techniques which gave equal (but not homologous) precipitin reactions, one may observe that in the last four columns of this table the elimination of the calcium oxalate reaction completely eliminates all inter-reactivity of the extracts employed. Hence one may conclude that the calcium oxalate reaction is the only one concerned here.

Summarizing, then, the experiments on extraction of the *Prunus*, *Platanus*, *Robinia*, and *Ribes* extracts, one may say that:

1. The reactive substances in these plants are insoluble in strong alcohol, ether, chloroform, carbon tetrachloride, benzol, and 95% alcohol + tartaric acid 1%.
2. They are not denatured by treatment with any of these solvents.
3. They are equally soluble in distilled water and physiological NaCl solution.
4. The reactions take place with equal facility in the presence of NaCl (.85%), MgO, and N/20 NaOH, and in acid and neutral solutions.

G. Effect of Carbohydrate Removal on the Reactions

In order to test the possibility that the reactive principles might be of carbohydrate nature, an experiment was undertaken to eliminate carbohydrates from consideration. The technique of Rimington (20) was employed. Normal extracts of *Platanus*, *Robinia*, and *Prunus* were prepared in the customary manner. To each of these was added a slight excess of neutral lead acetate. There was a voluminous precipitate, containing mucilaginous substances, etc. This precipitate was redissolved by washing and suspending in water and bubbling through H_2S. To the filtrate (neutral $Pb(C_2H_3O_2)_2$ filtrate) was then added about

1/10 the volume of the original extract of NH_4OH solution. A second voluminous precipitate resulted, this containing the bulk of the carbohydrates (alkaline PbAc precipitate). NH_4OH and $Pb(C_2H_3O_2)_2$ were then added in turn until the precipitate was maximum, and the filtrate (alkaline $Pb(C_2H_3O_2)_2$ filtrate) virtually negative to the Molisch test. The alkaline $Pb(C_2H_3O_2)_2$ precipitate was redissolved in acetic acid, and the excess lead was removed from it and from the alkaline $Pb(C_2H_3O_2)_2$ filtrate with H_2S. These solutions were then titrated back to slight acidity and the precipitin tests performed. The H_2S was removed by boiling, although tests showed that it did not interfere with the reaction. The result of these tests is given in Table XVII.

TABLE XVII.

EFFECT OF CARBOHYDRATE REMOVAL ON THE PRUNUS-PLATANUS-ROBINIA REACTION. [In this table PbAc = $Pb(C_2H_3O_2)_2$]

Control tests:

Prunus (Normal) + Robinia (Normal) = 3
" " + Platanus " = 3
Platanus " + Robinia " = 0

Tests for effect of H_2S on reaction:

Prunus (Normal + H_2S) + Robinia (Normal + H_2S) = 3
" " " + Platanus " " = 3
Platanus " " + Robinia " " = 0

		Prunus (Normal)	Platanus (Normal)	Robinia (Normal)
Robinia	Alk.PbAc filtrate,pH 5.2, Molisch test: trace	3	0	0
	Alk.PbAc precipitate, pH 4.8, Molisch test very strong	0	0	0
	Control (Normal extract)	3	0	0
Platanus	Alk.PbAc filtrate, pH 5.3, Molisch test very weak	3	0	0
	Alk.PbAc precipitate, pH 4.7, Molisch test very strong	0	0	0
	Control (Normal extract)	3	0	0
Prunus	Alk.PbAc filtrate, pH 5.8, 5.5, Molisch test weak	0,0	0,0	0,0
	Alk.PbAc precipitate, pH 5.0, 5.8, Molisch test very strong	0,0	0,0	0,0
	Neutral PbAc filtrate	0	0	0
	Neutral PbAc precipitate	0	3	3
	Control (Normal extract)	0	3	3

This experiment is of interest in distinguishing the reactive components of *Prunus* as opposed to those of *Platanus* and *Robinia*. The former are precipitated by neutral $Pb(C_2H_3O_2)_2$, the latter by neither neutral nor alkaline $Pb(C_2H_3O_2)_2$. The reaction appeared in full strength in a fraction showing only a trace of Molisch reaction and hence could not conceivably be due to a Molisch-carbohydrate in *Platanus* or *Robinia*. The fact that the active constituent of *Prunus* was precipitated by neutral $Pb(C_2H_3O_2)_2$ does not shed much light upon its nature, since this precipitate contains a highly heterogeneous mixture of substances, but it is of interest in view of the later findings regarding its oxalate nature, since many oxalates are precipitated but reclaimable by this technique.

H. Effect of Enzyme Digestion, Contamination, and Ageing on the Reactions

Of considerable interest is the question of whether the reactive ingredients of these extracts are affected by the action of enzymes. Indeed the failures of enzymes, suitably chosen and tested, to inactivate such reactive principles is very strong evidence against their protein nature. Accordingly the following experiments were planned to test this matter.

In the first experiment on this head, *Prunus*, *Platanus*, and *Robinia* were again used. Normal extracts of each of these species were titrated with KOH to pH 9.0-9.5 and then divided each into two portions. One portion of each was treated with a few drops of Difco Bacto-trypsin. Both portions of each were then covered with toluol and incubated at 37°C. Each day a few drops of the trypsin were added to each experimental tube. The incubation continued for 9 days. During this time no additional alkali was needed, as the rise in pH due to tryptic digestion was not over pH 1 during the experiment. That slight rise in pH which did take place was during the first three days alone. On removing from the oven the extracts all showed a greater or less precipitate, and this precipitate, for the most part, was not brought back into solution on acidification. The extracts were then acidified with HCl to their original pH, filtered or centrifuged, and used in testing. The results of these tests are shown in Table XVIII-A. Where two values are given for any given reaction (as "4, 4") this indicates two separate performances of the experiment, with extracts separately handled throughout. In the table "normal" signifies fresh, unheated extract, "control" signifies extracts titrated and incubated but not trypsinized.

As a second part to this experiment, 15 cc. each of normal extracts of the same three species were slightly acidified and treated with crystalline pepsin. The treated and control tubes were incubated at 37°C. for 8 days and then tested (Table XVIII-B).

For a third part to the same experiment, extracts of the same species were titrated to neutrality with KOH, placed in fermentation tubes at room temperature, and inoculated with baker's yeast. Control tubes remained beside them at room temperature. After five days all were cleared and tested. There was no gas production in any case. The results, (Table XVIII-C) being a duplication of those in part B, are not given in tabular form.

An examination of Table XVIII-A shows that in no case did the trypsin digestion result in a loss of reaction in any one of the three types of extract. The minor variations are not considered of signifi-

cance in view of the decided strength of all the positive reactions. The same is true of the trypsinized extracts (B) and of the extracts treated with yeast. One may conclude from this experiment that the reactive substances are highly resistant to the action of proteolytic enzymes and of the zymase complex, and accordingly the view of their non-protein nature is strengthened.

TABLE XVIII.

EFFECT OF ENZYME DIGESTION ON PRUNUS-PLATANUS-ROBINIA PRECIPITIN REACTION.

A. Effect of trypsin digestion.

		Prunus			Platanus			Robinia		
		Normal	Control	Exper.	Normal	Control	Exper.	Normal	Control	Exper.
Prunus	Normal	0	0	0	4	4	4,4	3	3	3,3
	Control	0	0	0	3	2	2	1	0	0
	Exper.	0	0	0	4,4	4	4,4	3,3	2	2,3
Platanus	Normal	4	3	4,4	0	0	0	0	0	0
	Control	4	2	4	0	0	0	0	0	0
	Exper.	4,4	2	4,4	0	0	0	0	0	0
Robinia	Normal	3	1	3,3	0	0	0	0	0	0
	Control	3	0	2	0	0	0	0	0	0
	Exper.	3,3	0	2,3	0	0	0	0	0	0

B. Effect of pepsin digestion.

Prunus....Normal	0	0	4	4	4	4
Prunus....Treated	0	0	4	4	4	4
Robinia...Normal	4	4	0	0	0	0
Robinia...Treated	4	4	0	0	0	0
Platanus..Normal	4	4	0	0	0	0
Platanus..Treated	4	4	0	0	0	0

C. Effect of yeast digestion. (Omitted. Results exactly as in "B" above)

Beside this experiment in enzyme digestion certain other evidence may be cited relative to the same question. Frequently extracts kept for long periods of time, particularly at room temperature or without protection with toluol, become contaminated with various types of microorganisms. Incidentally such extracts have frequently been reclarified and found still to retain strong precipitin potency. However, in order to have definite data to meet this question an experiment was arranged to illustrate the effect of contaminations on the extracts. In this experiment extracts of 6 species of *Nicotiana* were employed, all of which had become contaminated 3 days after they had been first prepared and tested. The contaminated extracts were cleared and tested again against the same solutions as had been used with the uncontaminated ones three days previously. The results of these tests are given in Table XIX.

It will be seen from an examination of this table that the contamination exerted no inhibitory effect upon the reactive substances, as there is no significant difference in the results of the tests before and after contamination. It should be noted here that the *Nicotiana* species all contained excess calcium and that they were tested against potassium oxalate simply because this gives an accurate measure of the calcium oxalate reaction. The significance of the test in no wise differs from that if *Prunus* had been used in place of the $K_2C_2O_4$. The tests against *N. Rusbyi,* on the other hand, are not due to the calcium oxalate reaction, as will be explained in the following sections, and for this reason the evidence on contamination of this experiment signifies the absence of its deleterious effect not only in the calcium oxalate reaction but in the MN reaction as well.

In addition to the experiments on enzyme digestion and on contamination, one other type of evidence should be considered in this section, namely the influence of age of the extracts. The question has seriously concerned the writers as to whether it was safe to use an extract, however clear, which had been preserved by cold and toluol for long periods of time. Accordingly an experiment to study the effect of age on the extracts was carried out.

A group of extracts (See Table XX) which had all been extracted and preserved at least four months were decanted and tested as is shown in the following table (Table XX).

An examination of the data shows that in no case was there any significant change attributable to the long period of storage. This fact indicates that future workers in this field may with confidence store extracts, at least of this type, for considerable periods of time at 2°C. and covered with toluol, and also that the auto-precipitations occurring in such stored extracts do not weaken the reactions.

I. Effect of Double Precipitation on the Reactions

A final point to consider in studying the analogy between the plant reactions and those of animals is the phenomenon of double precipitation. In serology the specificity of the precipitin reaction is such that if an antiserum A is prepared immune to two antigenic sera B and C, then A may be fully precipitated by B, cleared, and subsequently strongly precipitated by C. Presence of such a phenomenon in plants would strengthen the view that the plant reactions are homologous to those of animals, and absence of this phenomenon would reduce the plant reactions to a relatively simple type of precipitation.

In order to test this point, an experiment was planned involving

TABLE XIX.

EFFECT OF CONTAMINATION ON THE CALCIUM OXALATE AND MN REACTIONS. CON.: CONTAMINATED. UNCON.: UNCONTAMINATED.

	Precipitin reaction against the following species of Nicotiana:											
	alata		Langsdorffii		acuminata		Cavanillesii		sylvestris		glauca	
	Cont.	Uncon.	Con.	Uncon.	Con.	Uncon.	Con.	Uncon.	Con.	Uncon.	Con.	Uncon.
Nicotiana Rusbyi (M)	2	2	1	1	t	t	1	2	1	1	2	1
Potassium oxalate (M/100)	3	3	3	3	3	3	3	4	3	4	4	3

TABLE XX.

EFFECT OF STORING EXTRACTS FOR LONG PERIODS OF TIME IN THE COLD.

"a" = Test when extracted. "b" = Test after four months of storage.

		Prunus Armeniaca Mikado		Prunus Armeniaca Ansu		Photinia villosa		Deutzia scabra plena		Spiraea virginiana serrulata	
		"a"	"b"	"a"	"b"	"a"	"b"	"a"	"b"	"a"	"b"
Hydrangea panic.grfl.	"a"	2		2		0		0			
	"b"		2		2		0		0		
Photinia villosa	"a"	2		2		0					
	"b"		2		2		0				
Deutzia scabra pl.	"a"	t		t				0			
	"b"		1		1				0		
Pyracantha coccinea	"a"	2		2		0				0	
	"b"		2		2		0				0
Spiraea virg.serrul.	"a"	2		2						0	
	"b"		2		2						0

TABLE XXI.

DOUBLE PRECIPITATION IN THE PRUNUS-PLATANUS-ROBINIA-RIBES-HYDRANGEA REACTION COMPLEX.

	Tested against normal extracts of: Robinia	Platanus	Ribes	Hydrangea
Prunus control (Prunus : water = 1 : 4)....	2	2	3	3
Prunus + Robinia. Cleared mixture, 1 : 4...	0	0	0	0
Prunus + Platanus.Cleared mixture, 1 : 4...	0	0	0	0
Prunus + Ribes. Cleared mixture, 1 : 4...	0	0	0	0
Prunus + Hydrangea. Cleared mix., 1 : 4...	0	0	0	0
Robinia (Normal).....	0	0	0	0
Platanus (Normal).....	0	0	0	0
Hydrangea (Normal).....	0	0	0	0
Ribes (Normal).....	0	0	0	0

normal extracts of *Robinia, Platanus, Ribes, Hydrangea,* and *Prunus*. *Prunus* is positive to the other four, which latter four are negative *inter se*. As the *Prunus* control was prepared an extract consisting of one part normal *Prunus* extract + four parts water. The other extracts were prepared by mixing one part of normal *Prunus* extract with four parts of *Robinia, Ribes, Platanus,* or *Hydrangea* extracts respectively. A voluminous precipitate resulted in all cases. The latter was removed by centrifuging and the resulting cleared mixtures were tested as in Table XXI.

A consideration of these results shows that complete precipitation with an excess of any one of the four counter extracts completely eliminated any further reactivity of the *Prunus* with the other extracts. This is a situation very different from that of animal immunology, and points definitely to a qualitative difference between the plant and animal reactions. Indeed one is assured by a study of Table XXI that the reactive principle in *Prunus* is a single substance fully precipitable by any one of the other four extracts, which latter might well be assumed to contain the same reactive ingredient.

We have now reviewed the evidence regarding the analogy of the *Prunus-Platanus-Ribes-Robinia-Hydrangea* reactions to the zoöimmunitary precipitin reaction. Before passing on to the next subject it would be well briefly to enumerate the points demonstrated in the present section.

A. The reactive substances of the *Prunus-Platanus-Ribes-Robinia* complex differ from the specific protein reactions of animal sera in the following circumstances:
   1. The reactive substance of *Prunus* is manifestly different from that of *Platanus, Robinia, Ribes,* and *Hydrangea*. The reactive substances in these last four species, however, appear to be identical.
   2. The reactive substances in all extracts are highly resistant to heat, alcohol coagulation, variations in pH, enzyme digestion, coagulation by strong acids and alkalies, and bacterial and fungus contaminations.
   3. The reactive substances in all these extracts pass freely through dialyzing membranes impermeable to formed protein but permeable to crystalloids.
   4. There is no evidence of a zone phenomenon in these extracts comparable to that of animal serology.

B. The reactive substances are non-lipoid in nature.

C. They are, at least in some cases, non-carbohydrate in nature.

D. They are unaffected by any of Silberschmidt's various techniques of pre-extraction and solution, including pre-extraction with alcohol, ether, and chloroform, and solution in water, physiological saline (acid or neutral), and N/20 NaOH.

IV. PROOF OF THE CALCIUM OXALATE REACTION

The preceding section having treated with a comparison of the plant precipitin reaction with that of animal immunology and having dealt with the evidence that the plant reaction is non-protein, non-lipoid, and non-carbohydrate, the present section will concern itself with a proof of the exact nature of the reaction in the *Prunus-Platanus-Robinia-Ribes* complex and an extension of this proof to a variety of other plants.

The first successful line of attack of this problem was in experiments in ignition of extracts and precipitates. It has already been pointed out (page 125) that a preliminary experiment in the ignition of extracts failed to reveal the reactive substances in solutions of the ash. However, a new type of experiment was resorted to in order to determine whether the precipitate itself was of organic or inorganic nature.

Clear normal extracts were prepared of *Prunus*, *Platanus*, *Robinia*, and *Ribes* in the ordinary way. These were centrifuged for several minutes to throw down any further organic débris. Then the following mixtures were placed in centrifuge tubes:

Prunus (5 cc.) + *Platanus* (5 cc.)
" " + *Robinia* (5 cc.)
" " + *Ribes* (5 cc.)

These were permitted to interact for 10 minutes, and were then centrifuged. Each precipitate from centrifuging was washed in three changes of distilled water, suspending the precipitate in water and centrifuging after each change. The precipitates were then suspended in the last water and each was ignited in a small beaker at dull red heat (500°C.). In no case was there evidence of any appreciable amount of carbon in the process of ignition. The deposits remained perfectly white throughout, with only the slightest trace of pale brown momentarily appearing. This experiment was subsequently repeated several times but always with the same result.

A second line of attack was the solubilities of the precipitates. It was found that these were highly insoluble in all ordinary solvents. In fact solution was successful only in strong acid, while alcohol, lipoid solvents, boiling water, benzol and toluol, weak acids, and alkalies all failed to dissolve them.

At this point qualitative analysis of the precipitates was resorted to. The analysis gave the following results:

1. In physical appearance the precipitates resembled organic solids in being limey, granular, heavy, and easily centrifuged.
2. Qualitative analysis showed calcium and oxalate ions to be the only ones present in any appreciable quantity.
3. Microscopically the precipitates were in the form of regular granules, not amorphous, identical in appearance with certain commercial samples of calcium oxalate.
4. Recrystallization of the precipitates (by solution in 6N H_2SO_4 followed by precipitation by neutralization with 4N KOH) gave an abundance of crystals of the characteristic size and shape of CaC_2O_4 crystals and indistinguishable from crystals of a commercial sample of CaC_2O_4 similarly treated.
5. Treatment of the granules of the precipitates with 6N H_2SO_4 under the microscope showed first a moderate solution followed by a very striking conversion of the remainder of the granules into the characteristic raphides of $CaSO_4$. This is a fairly accurate test for CaC_2O_4, and was precisely the behavior of a sample of commercial CaC_2O_4 similarly treated.
6. When alcohol was added to the H_2SO_4 solution of the precipitates, there was a precipitation (test for calcium).
7. The acid solution of the precipitates strongly reduced potassium permanganate (test for oxalates).

Next, through the courtesy of A. D. Bliss of the Harvard chemistry laboratories, a quantitative analysis was made of specimens of the washed precipitate prepared as was that in the ignition experiments described above. Mr. Bliss' analysis of the precipitate gave the following results.

The preliminary identification of the precipitate as calcium oxalate on the basis of its qualitative reactions, etc., was well confirmed by the quantitative analytical data, for in view of the stoichiometric relationships involved nothing else could be present to any great extent. The small amount of substance available naturally rendered extensive analyses impossible, but the calcium content was determined and also the permanganate-reducing power of the organic radical involved.

To determine the calcium, a specimen was weighed, ignited at red heat, then cooled and weighed. Calculating the weight of calcium oxalate equivalent to the calcium oxide obtained, allowing for the fact that when prepared from wet materials and in contact with moist air calcium oxalate exists as the monohydrate, $CaC_2O_4.H_2O$, the result was 85.89% of calcium oxalate in the material.

Since the calcium oxalate might not be pure, it was next dissolved in HCl and the calcium precipitated with ammonium oxalate in ammoniacal solution. The calcium oxalate was filtered off, washed, and ignited to constant weight as the oxide. The product was pure white, as it should have been. With the weight of calcium oxalate thus obtained the calculated amount of calcium oxalate was 84.61%. This percentage does not differ very much from the first, showing that the oxide from the first ignition was practically pure calcium oxide.

The percentage of calcium in the original sample was then found to be 23.22% as compared with 27.43% in pure calcium oxalate. This discrepancy may have been due to one or more of several causes: (1) presence of metals other than calcium, (2) other organic aids than oxalic, of higher molecular weight, or (3) organic matter which would disappear on ignition.

In order to check the results of the calcium determination, a titration of the reducing power of the material was made with standard permanganate solution. This titration gave for the percentage of calcium oxalate in the precipitate 87.94%. The difference between this value, 87.94%, and that found from the calcium analysis, 84.61% may have been due in part to the normal errors of analysis, to a possible difference in composition of the material, since the two samples were independently obtained, and lastly to higher molecular weight acid radicals or organic débris in the sample, as mentioned above.

The qualitative and quantitative analyses having agreed as to the calcium oxalate nature of the precipitates from the *Prunus-Platanus-Robinia-Ribes* reactions, the next step was to determine the behavior of extracts of these and other plants in the presence of solutions of calcium and oxalate. The first test of this type was with normal extracts of *Prunus*, *Platanus*, and *Robinia* as tested against one another and against certain calcium compounds as well as certain oxalates. These tests were all performed in the customary manner, and the results are given in the following table (Table XXII).

A study of this table shows that *Prunus* behaves exactly as do the oxalates, while *Platanus* and *Robinia* behave in the same fashion as the calcium salts.

This same type of analysis was next applied to a variety of all the woody plants which were at the time available. All were tested against potassium and ammonium oxalate and calcium chloride and nitrate as well as against one another (Table XXIII). All the plants were found to fall into two groups, one group including only *Prunus* which was negative to the oxalates and positive to the calcium salts, the other

TABLE XXII.

RELATION OF THE PRUNUS-ROBINIA-RIBES REACTION TO THE PRESENCE AND ABSENCE OF CALCIUM AND OXALATE.

	Parts soluble in 100 parts of water:	Ribes, normal extract:	Robinia, normal extract:	Prunus, normal extract:	Potassium oxalate:	Ammonium oxalate:
Calcium nitrate	93.	0	0	2	2	2
Calcium chloride	70.	0	0	2	2	2
Calcium acetate	42.	0	0	2	2	2
Calcium sulphate	.2	0	0	1	1	1
Calcium tungstate	.2	0	0	0	0	0
Calcium phosphate	Trace	0	0	0	0	0
Calcium fluoride	Trace	0	0	0	0	0
Potassium oxalate	33.	2	2	0	0	0
Ammonium oxalate	4.2	2	2	0	0	0

TABLE XXIII.

PRESENCE OR ABSENCE OF PRECIPITIN REACTIONS IN CERTAIN WOODY PLANTS IN RELATION TO PRESENCE OR ABSENCE OF CALCIUM AND OXALATE.

	Potassium oxalate	Ammonium oxalate	Prunus Armeniaca	Platanus acerifolia	Robinia fertilis	Ribes Carrieri	Hydrangea paniculata	Syringa vulgaris	Ligustrum obtusifolium	L. vulgare	L. ibota	Calcium chloride	Calcium nitrate
Potassium oxalate	0	0	0	+	+	+	+	+	+	+	+	+	+
Ammonium oxalate	0	0	0	+	+	+	+	+	+	+	+	+	+
Prunus Armeniaca	0	0	0	+	+	+	+	+	+	+	+	+	+
Platanus acerifol.	+	+	+	0	0	0	0	0	0	0	0	0	0
Robinia fertilis	+	+	+	0	0	0	0	0	0	0	0	0	0
Ribes Carrieri	+	+	+	0	0	0	0	0	0	0	0	0	0
Hydrangea panicul.	+	+	+	0	0	0	0	0	0	0	0	0	0
Syringa vulgaris	+	+	+	0	0	0	0	0	0	0	0	0	0
Lig. obtusifolium	+	+	+	0	0	0	0	0	0	0	0	0	0
L. vulgare	+	+	+	0	0	0	0	0	0	0	0	0	0
L. ibota	+	+	+	0	0	0	0	0	0	0	0	0	0
Calcium chloride	+	+	+	0	0	0	0	0	0	0	0	0	0
Calcium nitrate	+	+	+	0	0	0	0	0	0	0	0	0	0

group containing the remainder of the woody species, which were perfectly negative *inter se* and to the calcium salts but all positive to the oxalates and to *Prunus*.

We thus see that all of the reactions observed in the woody plants are susceptible to explanation on the basis of a calcium oxalate precipitation occurring as a result of the interaction of oxalate ions in *Prunus* with calcium ions in the remainder of the extracts. No other hypothesis is necessary to explain any of the reactions in this group of plants, and that the reactions observed are due to calcium oxalate is manifest not only from the analytical studies above but also from the simple chemical fact that the tests of these species with calcium salts and with oxalates (Table XXIII) demonstrates that they must contain calcium or oxalate as indicated. Furthermore it follows from the chemistry of such ions that if one adds a solution containing free calcium to one containing free oxalate, a precipitate must result. We thus find that not only *do* these precipitations occur between the extracts under consideration, but that they *must* occur from the chemical laws governing the behavior of calcium in the presence of an oxalate.

It was now felt desirable to extend these results to a greater variety of plants to observe the extent of distribution and the importance of the calcium oxalate reaction in plants, particularly in those in which much of the work on the precipitin reaction has been accomplished. Accordingly the same type of experiment as that of Table XXIII was applied to a variety of species of the *Solanaceae*. The results of these tests are gathered and arranged in Table XXIV.

A glance at this table will assure one that the same factors are at work here as were observable in the woody plants. Of the 21 species chosen at random, 8 were negative to the oxalates and positive to the calcium salts, 10 others were negative to the calcium salts and positive to the oxalates. The 8 "oxalate" extracts were all in varying degree positive to the 10 "calcium" extracts. Three extracts, *Cyphomandra*, *Browallia*, and *Datura Wrightii*, were negative or practically so to both salts, and were correspondingly negative to both the "calcium" and "oxalate" extracts. Moreover the "oxalate" extracts were perfectly negative *inter se*, while the "calcium" extracts were nearly so. Thus the same remarks as were applied to the results within the woody plants under consideration apply equally to the *Solanaceae*. The calcium oxalate reaction is of frequent occurrence in the *Solanaceae* as it was in the woody plants, almost all of the reactions in Table XXIV being explainable in terms of calcium oxalate precipitations, and moreover the behavior of these extracts with the pure solutions of the salts requires that

such "precipitin reactions" take place when the various pairs of extracts are combined.

In observing the results in Table XXIV it will be seen that the "calcium" extracts are not perfectly negative *inter se*. However, the few reactions which do appear in this block of the table will be explained in the following section.

TABLE XXIV.

PRECIPITIN REACTIONS AND CALCIUM OXALATE REACTIONS OF CERTAIN SOLANACEAE.

	Ammonium oxalate .02N	Potassium oxalate .01N	Atropa Belladonna	Datura ferox	Solanum tuberosum	Capsicum frutescens	Datura metel	D. innoxia	Physalis Peruviana	Salpiglossis sinuata	Cyphomandra betacea	Browallia viscosa	Datura Wrightii	Nicotiana Rusbyi	N. paniculata	Petunia violacea	Lycopersicum cerasiforme	Solanum Capsicastrum	Nicotiana rustica	N. suaveolens	N. Tabacum	Solanum nigrum	S. melongena	Calcium chloride .005N	Calcium nitrate .005N
Ammonium oxalate .02N	0	0	0	0	0	0	0	0	0	0	0	0	0	t	t	1	2	1	2	2	2	2	2	1	1
Potassium oxalate .01N	0	0	0	0	0	0	0	0	0	0	0	t	t	1	2	1	1	2	2	2	2	2	3	2	2
Atropa Belladonna	0	0	0	0	0	0	0	0	0	0	0	t	0	2	3	3	3	2	3	3	3	3	3	3	3
Datura ferox	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	3	2	2	2	3	2	2	2	2	3
Solanum tuberosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	2	4	2	3	2	2	1	2
Capsicum frutescens	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	2	2	1	3	2	3	3	2	2	1
Datura metel	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	2	1	3	2	3	1	1	1	2
Datura innoxia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	3	1	2	3	2	1	1	1	1
Physalis Peruviana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	2	2	2	2	2	2	1	1	1
Salpiglossis sinuata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	1	2	0	0	1	1
Cyphomandra betacea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	t	t	t	0	0	0	0
Browallia viscosa	0	t	t	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Datura Wrightii	0	t	0	0	0	0	0	0	0	0	0	0	0	t	t	0	t	0	0	0	t	0	0	0	0
Nicotiana Rusbyi	t	1	2	0	0	2	0	0	0	0	0	0	t	0	0	0	0	1	1	1	1	0	t	0	0
N. paniculata	t	2	3	3	2	3	3	3	2	0	0	0	t	0	0	0	0	0	0	0	0	0	0	0	0
Petunia violacea	1	1	3	3	2	2	2	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lycopersicum cerasiforme	2	1	3	2	1	2	2	3	2	t	0	0	t	0	0	0	0	0	0	0	0	0	0	0	0
Solanum Capsicastrum	1	2	2	2	2	1	1	1	2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Nicotiana rustica	2	2	3	2	4	3	3	2	2	2	t	0	0	1	0	0	0	0	0	0	0	0	0	0	0
N. suaveolens	2	2	3	3	2	2	2	3	2	1	t	0	0	1	0	0	0	0	0	0	0	0	0	0	0
N. Tabacum	2	2	3	2	3	3	3	2	2	2	t	0	t	1	0	0	0	0	0	0	0	0	0	0	0
Solanum nigrum	2	2	3	2	2	3	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. melongena	2	3	3	2	2	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Calcium chloride .005N	1	2	3	2	1	2	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Calcium nitrate .005N	1	2	3	3	2	1	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

We may thus conclude this section with the following brief statement of the occurrence of the calcium oxalate reaction: The reactions between *Prunus*, *Platanus*, *Robinia*, and *Ribes* have been proved to be due to the presence in *Prunus* of free oxalate which combines with free calcium in the other extracts to produce a precipitation of calcium oxalate. The existence of free calcium and free oxalate in various other

woody plants as well as in a variety of the *Solanaceae* has been demonstrated, and as a necessary corollary, calcium oxalate precipitations must occur whenever two extracts containing these two ions respectively are mixed. Indeed all the precipitations observed in the preceding tables in the woody plants and nearly all in the *Solanaceae* were precipitates of calcium oxalate.

Mentally reviewing the evidence on the nature of the reaction presented in the earlier part of this paper, all that has been obtained is in perfect agreement with this conception, the relative insensitivity to pH, heat, enzymes, and alcohol, the effect of increasing salt concentration, the regular behavior in dilution, the readiness with which the reactive principles pass through dialyzing membranes, their insolubility in alcohol and lipoid solvents, and their identity in the experiments in double precipitation, all are in agreement with the calcium oxalate interpretation.

However, one very important question remains: Is the calcium oxalate reaction sufficient to account for *all* of the reactions observed? The answer to this question will be considered in the following section.

V. PROOF OF THE EXISTENCE OF OTHER REACTIONS

At this stage in the development of the problem, it was felt that a very important confirmation of the calcium oxalate explanation would result from the behavior of the "calcium" woody extracts in the presence of the "calcium" solanaceous extracts. The number of *Solanaceae* was increased to 36 species for this experiment, and the precipitin reactions of all the possible combinations of these 42 species were determined. In addition, all the extracts were tested against two oxalates and against two calcium salts. The species were then arranged according to their reactivity with the salts and tabulated as in Table XXV.

Again the 42 species fall into three classes, a "calcium" class, an "oxalate" class, and a class reactive to neither. The "oxalate" class (Ca—, Ox+) is negative *inter se,* as would have been predicted; so also is the (Ca—, Ox—) class. The "calcium" class (Ca+, Ox—) are all positive to the Ca—, Ox+) class and reciprocally. Thus far the calcium oxalate reaction is perfectly sufficient for our explanation. But when one concentrates on the inter-reactions of the members of the (Ca+, Ox—) class there are many positive reactions unaccounted for. These, however, are not irregularly distributed through the table but appear to be the reactions with certain specific extracts, notably *Robinia, Platanus,* and *Ribes.* Moreover the strength of reaction of any given solanaceous extract is proportional toward all of the three

woody extracts mentioned. It is thus clear that there is another reaction present, occurring between the woody species and the majority of the *Solanaceae*. This reaction can be studied best by eliminating

TABLE XXV.

A COMPREHENSIVE TABLE OF ALL THE PRECIPITIN REACTIONS IN THE PLANTS EMPLOYED IN THIS STUDY, EMPHASIZING PARTICULARLY THE CALCIUM OXALATE REACTION.

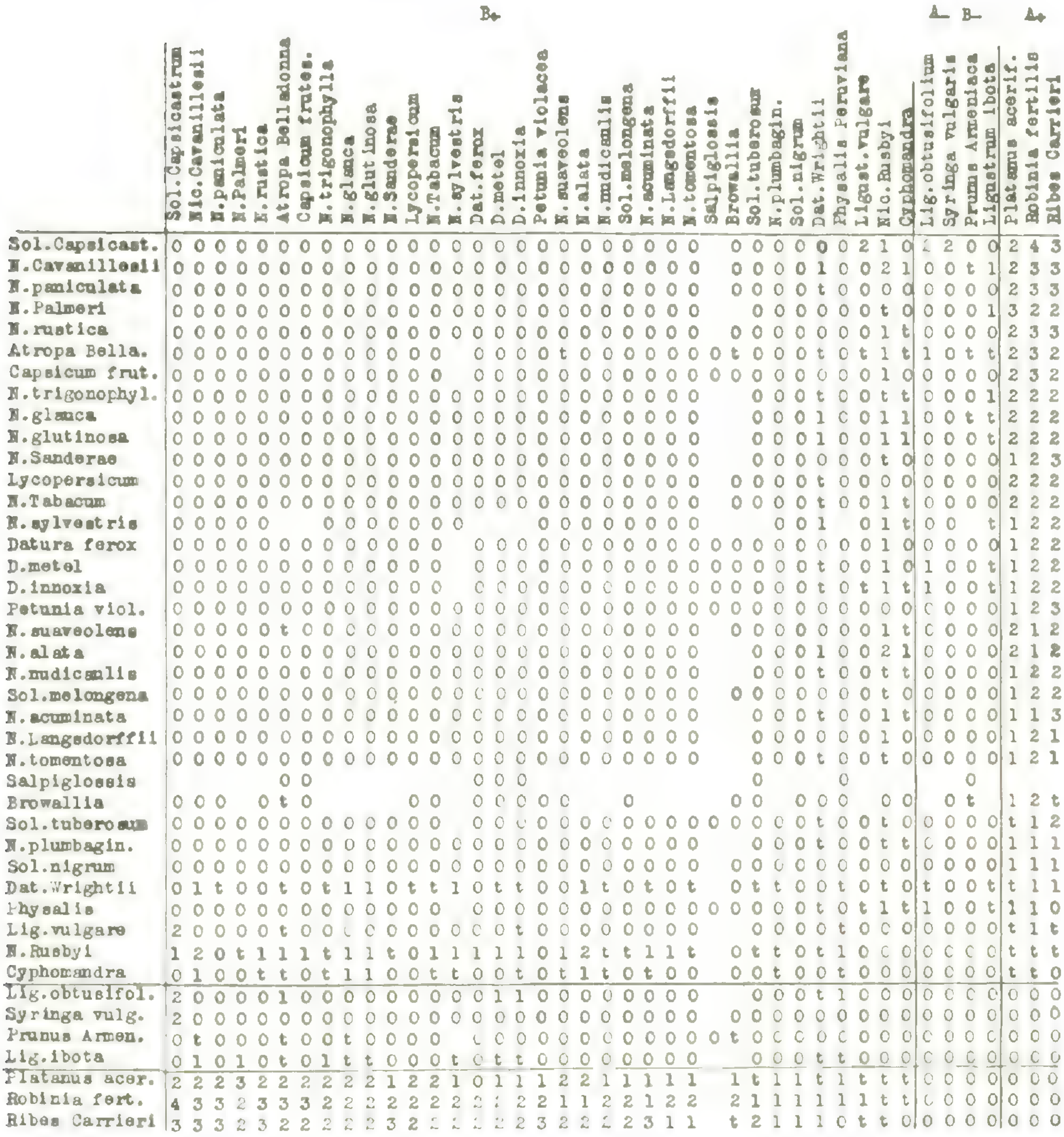
	Ca- Ox+									CaOx-			Ca + Ox -																													
	Atropa Bella.	Dat.ferox	Prunus	Sol.tuberos.	Capsicum	Dat.metel	Physalis	Dat.innoxia	Salpiglossis	Cyphomandra	D.Wrightii	Browallia	Lig.vulgare	L.obtusifol.	Nic.Rusbyi	Petunia	Robinia	Platanus	Ribes	N.paniculata	N.Palmeri	N.trigonoph.	N.Sanderae	Lycopersicum	Sol.Capsic.	N.plumbagin.	Lig.ibota	Syringa	Sol.nigrum	N.suaveolens	N.alata	N.acuminata	N.glauca	N.glutinosa	N.nudicaulis	N.Langsdorff.	N.tomentosa	N.Tabacum	N.Cavanill.	N.rustica	N.sylvestris	Sol.melongena
Atropa	0	0	t	0	0	0	0	0	0	0	0	t	1	1	2	3	3	2	2	3	1	1	1	3	2	2	1	2	3	3	3	1	3	2	2	1	1	3	2	3	2	3
Datura ferox	0	0	t	0	0	0	0	0	0	0	0	0	1	1	0	3	2	2	2	3	2	1	1	2	2	2	1	2	2	3	3	2	2	2	2	1	2	2	3	2	2	2
Prunus Armen.	t	t	0	0	0	0	0	0	0	0	t	t	2	2	1	2	2	1	1	3	t	1	2	2	2	1	2	3	2	3	2	3	2	2	2	2	1	4	3	3	2	2
Sol.tuberos.	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2	1	2	3	2	2	2	1	1	2	1	1	2	2	2	3	1	3	2	3	1	2	3	2	4	2	2
Capsicum	0	0	0	0	0	0	0	0	0	0	0	0	t	1	2	2	2	2	2	3	1	t	1	2	1	t	1	1	3	2	2	1	2	2	2	t	1	3	1	3	2	2
Dat.metel	0	0	0	0	0	0	0	0	0	0	0	0	1	t	0	2	2	2	2	3	1	2	1	2	1	1	1	1	1	2	2	2	2	2	3	1	1	3	1	3	2	1
Physalis	0	0	0	0	0	0	0	0	0	0	0	0	t	1	0	1	1	2	1	2	1	1	1	2	2	1	2	1	2	2	2	1	2	2	2	2	2	2	2	2	2	1
Dat.innoxia	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	2	1	2	2	3	1	1	1	3	1	1	t	1	1	3	2	2	2	2	2	1	1	2	1	2	1	1
Salpiglossis	0	0	0	0	0	0	0	0	0	0	0	0			0	1	1	2	1	0				0	0			0	0	1								2		2		0
Cyphomandra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	t	t	0	0	0	t	0	0	0	t	0	0	0	t	1	t	1	1	t	0	0	t	1	t	t	0
Dat.Wrightii	0	0	t	0	0	0	0	0	0	0	0	0	0	0	t	0	1	t	t	t	0	t	0	t	0	t	t	0	0	0	1	t	1	1	t	0	t	t	1	0	1	0
Browallia	t	0	t	0	0	0	0	0	0	0	0	0			0	0	2	1	t	0				0	0			0	0	0								0		0		0
Lig.vulgare	1	1	2	1	t	1	t	1		0	0		0	0	0	0	1	t	t	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L.obtusifol.	1	1	2	1	1	t	1	2		0	t		0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nic.Rusbyi	2	0	1	0	2	0	0	0	0	0	t	0	0	0	0	0	t	t	t	0	t	t	t	0	1	t	0	0	0	1	2	t	1	1	t	1	t	1	2	1	1	t
Petunia	3	3	2	2	2	2	1	2	1	0	0	0	0	0	0	0	2	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Robinia	3	2	2	1	2	2	1	1	1	t	1	2	1	0	t	2	0	0	0	3	2	2	3	2	4	1	0	0	1	1	1	1	2	2	2	2	2	2	3	2	2	1
Platanus	2	2	1	2	2	2	2	2	2	t	t	1	t	0	t	1	0	0	0	2	3	2	1	2	2	1	0	0	1	2	2	1	2	2	1	1	1	2	2	2	1	1
Ribes Carr.	2	2	1	3	2	2	1	2	1	0	1	t	t	0	t	2	0	0	0	3	2	2	2	2	3	1	0	0	1	2	2	3	2	2	2	1	1	2	3	3	2	2
N.paniculata	3	3	3	2	3	3	2	3	0	0	t	0	0	0	0	0	3	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.Palmeri	1	2	t	2	1	1	1	1		0	0		0	0	t	0	2	3	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.trigonoph.	1	1	1	2	t	2	1	1		t	t		0	0	t	0	2	2	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.Sanderae	1	1	2	1	1	1	1	1		0	0		0	0	t	0	3	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lycopersicum	3	2	2	1	2	2	2	3	0	0	t	0	0	0	0	0	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sol.Capsic.	2	2	2	2	1	1	2	1	0	0	0	0	2	2	1	0	4	2	3	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.plumbagin.	2	2	1	1	t	1	1	1		t	t		0	0	t	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lig.ibota	1	1	2	1	1	1	2	t		0	t		0	0	0	0	0	0	0	0	1	1	0	0	2	0	0	0	0	0	0	0	t	t	0	0	0	0	1	0	t	0
Syringa vulg.	2	2	3	2	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sol.nigrum	3	2	2	2	3	1	2	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.suaveolens	3	3	3	2	2	2	2	3	1	t	0	0	0	0	1	0	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.alata	3	3	2	3	2	2	2	2		1	1		0	0	2	0	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.acuminata	1	2	2	1	1	2	1	2		t	t		0	0	t	0	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.glauca	3	2	2	3	2	2	2	2		1	1		0	0	1	0	2	2	2	0	0	0	0	0	0	0	t	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.glutinosa	2	2	2	2	2	2	2	2		1	1		0	0	1	0	2	2	2	0	0	0	0	0	0	0	t	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.nudicaulis	2	2	2	3	2	3	2	2		t	t		0	0	t	0	2	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.Langsdorff.	1	1	2	1	t	1	2	1		0	0		0	0	1	0	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.tomentosa	1	2	1	2	1	1	2	1		0	t		0	0	t	0	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.Tabacum	3	2	4	3	3	3	2	2	2	t	t	0	0	0	1	0	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.Cavanill.	2	3	3	2	1	1	2	1		1	1		0	0	2	0	3	2	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.rustica	3	2	3	4	3	3	2	2	2	t	0	0	0	0	1	0	2	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.sylvestris	2	2	2	2	2	2	2	1		t	1		0	0	1	0	2	1	2	0	0	0	0	0	0	0	t	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sol.melongena	3	2	2	2	2	1	1	1	0	0	0	0	0	0	t	0	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Calc.chloride	3	2	2	1	2	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Calc.nitrate	3	3	2	2	1	2	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potas.oxalate	0	0	0	0	0	0	0	0	0	0	t	t	1	1	1	1	1	1	1	2	2	2	2	1	2	2	1	2	2	2	3	3	3	3	3	3	3	2	4	2	4	3
Ammon.oxalate	0	0	0	0	0	0	0	0	0	0	0	0	t	t	t	1	1	1	1	t	t	t	t	2	1	1	t	2	2	2	1	1	1	1	1	1	1	2	t	2	1	2

entirely all of the calcium oxalate reactions. Such elimination automatically occurs in the interreactions of the (Ca+, Ox—) group and can be made to extend to all of the extracts in Table XXV by adding a slight excess of calcium to the (Ca—, Ox+) extracts and filtering.

Such was done, and the precipitin reactions of all these species were determined anew. They are tabulated in Table XXVI, the arrangement of species being in order of their decreasing reactivity toward

TABLE XXVI.

ALL THE PRECIPITIN REACTIONS IN THE PLANTS STUDIED AFTER ELIMINATION OF THE CALCIUM OXALATE REACTION. EMPHASIZING THE AB REACTION.



Robinia, *Platanus*, and *Ribes*, which latter are plainly similar with regard to this reaction.

We now find that we are able to separate all the extracts into three groups, one group (A—, B+) which is positive toward the three woody species, a second group (A+, B—) which is composed of these three

woody species, and a third group (A—, B—) which is negative to both the former groups. Assuming the existence of two reactive principles, A in *Platanus*, *Ribes*, and *Robinia*, B in the *Solanaceae*, and neither in the *Oleaceae*, A reacting with B to produce a precipitate, then nearly all of the reactions are accounted for. This AB reaction, the existence of which later experimentation has further confirmed, thus easily accounts for the majority of the reactions not reduceable to calcium oxalate.

A study of Table XXVI but eliminating from consideration the reactions of *Platanus*, *Robinia*, and *Ribes* is of interest in the following connection. If the calcium oxalate and AB reactions were the only reactions present in this group of extracts, all the remaining combinations should give wholly negative results, since they all lack both oxalate and principle A. Such is nearly but not entirely the case. There are a few reactions remaining, particularly those of *Nicotiana Rusbyi*, *Datura Wrightii*, and *Cyphomandra*. A reorganization of the data in Table XXVI so as to include only the oxalate-negative, A-negative extracts, and arranged according to their reactivity with *Nicotiana Rusbyi* gives the interesting situation of Table XXVII.

The reactions of *Datura Wrightii* and *Cyphomandra* are plainly correlated with those of *N. Rusbyi*, as possibly are those of *Prunus* and *Ligustrum ibota*. The table clearly points to a third pair of reactive substances, M in the species just mentioned which precipitates in the presence of N, present in most of the *Solanaceae* but absent in *Solanum nigrum*, *Ligustrum vulgare*, *Syringa*, *Petunia*, *Browallia*, and *Salpiglossis*.

The calcium oxalate, AB, and MN reactions together account for all but a very small percentage of the reactions observed in these species. There still remain, however, a few scattered reactions of which those of *Ligustrum obtusifolium* are most striking. Rearranging, then, the remaining oxalate-negative, A-negative, M-negative extracts as nearly as possible in order of their reactivity with the *Oleaceae*, we obtain Table XXVIII.

Table XXVIII assumes the presence of a reactive principle Y present in the *Oleaceae* considered, lacking in all the other plants, and precipitating in the presence of X which is found in certain of the *Solanaceae* (*Datura metel*, *D. innoxia*, *Physalis*, *Atropa*, and *Solanum Capsicastrum*).

This scheme accounts for every one of the reactions observed in these 42 species of plants. It is, moreover, confirmed by another type of experiment which will be here briefly mentioned.

It will be recalled (page 156) that when *Prunus* extract was thoroughly precipitated with *Platanus*, *Ribes*, *Robinia*, or *Hydrangea*, the resulting clear mixture was perfectly negative to the remaining woody

TABLE XXVII.

PRECIPITIN REACTIONS IN THE PLANTS STUDIED AFTER ELIMINATION OF THE CALCIUM-OXALATE AND AB REACTIONS. EMPHASIZING THE MN REACTION.

															N+																MN-						M+		
	Nic.alata	N.Cavanillesii	N.glauca	N.glutinosa	N.sylvestris	N.Tabacum	Atropa Bella.	N.acuminata	N.rustica	Datura metel	N.trigonoph.	Dat.innoxia	N.suaveolens	Physalis	N.nudicaulis	N.plumbagin.	Sol.Capsic.	Datura ferox	N.Langsdorffii	Capsicum	N.tomentosa	Sol.tuberosum	N.paniculata	N.Palmeri	N.Sanderae	Lycopersicum	Sol.melongena	L.obtusifol.	Sol.nigrum	Lig.vulgare	Syringa vulg.	Petunia viol.	Salpiglossis	Browallia	Prunus Armen.	Lig.ibota	Cyphomandra	Dat.Wrightii	N.Bushyi
Nic.alata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	1	1	2
N.Cavanillesii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	t	1	1	1	2
N.glauca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			t	t	1	1	1
N.glutinosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	t	1	1	1
N.sylvestris	0	0	0	0	0	0		0	0		0		0	0	0	0	0		0		0		0	0	0	0	0	0	0	0	0	0				t	t	1	1
N.Tabacum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	t	t	1
Atropa Bella.	0	0	0	0		0	0	0	0	0	0	0	t	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	t	0	0	0	t	t	t	t	t	1
N.acuminata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	t	t	1
N.rustica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	t	0	1
Datura metel	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	t	0	t	1
N.trigonoph.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	1	t	t	t
Dat.innoxia	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	t	0	0	0	0	0	t	t	t	1
N.suaveolens	0	0	0	0	0	0	t	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	t	0	1
Physalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	t	0	0	0	0	0	t	t	t	1
N.nudicaulis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	t	t	t
N.plumbagin.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	t	t	t
Sol.Capsic.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	2	0		0	0	2	0	0	1
Datura ferox	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
N.Langsdorffii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	0	1
Capsicum	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
N.tomentosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	t	t
Sol.tuberosum	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	t	t
N.paniculata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	t	0
N.Palmeri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	1	0	0	t
N.Sanderae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	0	t
Lycopersicum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	t	0
Sol.melongena	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	t
L.obtusifol.	0	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	t	0
Sol.nigrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0
Lig.vulgare	0	0	0	0	0	0	t	0	0	0	0	t	0	t	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	0	0
Syringa vulg.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0
Petunia viol.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0
Salpiglossis										0		0		0				0		0		0											0		0				
Browallia		0				0	t		0	0		0	0	0			0	0		0		0	0			0	0		0		0	0		0	t		0	0	0
Prunus Armen.	0	t	t	0		0	t	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	t	0	0	0	0	0
Lig.ibota	0	1	t	t	t	0	t	0	0	t	1	t	0	t	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0			0	0	0	t	0
Cyphomandra	1	1	1	1	t	t	t	t	t	0	t	t	t	t	t	t	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0
Dat.Wrightii	1	1	1	1	1	t	t	t	0	t	t	t	0	t	t	t	0	0	0	0	t	t	t	0	0	t	0	t	0	0	0	0		0	0	t	0	0	t
N.Bushyi	2	2	1	1	1	1	1	1	1	1	t	1	1	1	t	t	1	1	1	1	t	t	0	t	t	0	t	0	0	0	0	0		0	0	0	0	t	0

plants. When, on the other hand, *Prunus* was allowed thoroughly to interact with *Ribes*, then cleared, the resulting clear mixture was precipitated strongly by *Petunia*, *Lycopersicum*, *Nicotiana Tabacum*, *N. rustica*, and *N. paniculata*. This accordingly offers additional evidence as to the presence of a second reaction, in this case the AB reaction.

There is, then, no doubt that beside the calcium oxalate reaction there exist certain other reactions in this group of plants. These additional reactions have been tentatively referred to as the AB, MN, and

TABLE XXVIII.

PRECIPITIN REACTIONS IN THE PLANTS STUDIED AFTER ELIMINATION OF THE CALCIUM OXALATE, AB, AND MN REACTIONS. EMPHASIZING THE XY REACTION.

	Y+ L.obtusifol.	L.vulgare	Syringa vulg.	XY- Browallia	N.suaveolens	N.Cavanillesii	N.glauca	N.glutinosa	N.sylvestris	N.tabacum	N.acuminata	N.rustica	N.trigonophylla	N.alata	N.nudicaulis	N.plumbagin.	N.Langsdorffii	N.tomentosa	N.paniculata	N.Palmeri	N.Sanderae	Capsicum	Lycopersicum	Petunia	Salpiglossis	Datura ferox	Sol.tuberosum	S.melongena	S.nigrum	X+ Datura metel	D.innoxia	Physalis	Atropa	S.capsicastrum
L.obtusifol.	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	2
L.vulgare	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	t	t	t	2
Syringa vulg.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Browallia			0	0	0	0			0		0											0		0	0	0		0	0	0	0	0	0	0	0
Nic.suaveol.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.Cavanilles.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.glauca	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.glutinosa	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.sylvestris	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0		0
N.tabacum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.acuminata	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.rustica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.trigonophy.	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.alata	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.nudicaulis	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.plumbagin.	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.Langsdorfii	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.tomentosa	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.paniculata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.Palmeri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N.Sanderae	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Capsicum	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lycopersicum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Petunia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salpiglossis	0	0																				0		0		0	0	0		0	0	0	0	
Datura ferox	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S.tuberosum	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S.melongena	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sol.nigrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Datura metel	1	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D.innoxia	1	t	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Physalis	1	t	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Atropa	1	t	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S.capsicast.	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

XY reactions. What is their nature? It will be the purpose of the following section to answer this question as fully as the experimental data permit, although it must be freely confessed that the investigation in this direction has only begun.

VI. PROPERTIES OF THE AB, MN, AND XY REACTIONS

A. Heat Experiments

The AB reaction, according to our analysis of the preceding section, results from the interaction of a substance A present in *Robinia*, *Platanus*, and *Ribes* with a substance B present generally in the *Solanaceae* but absent in the *Oleaceae*. The MN reaction results in a precipitation from the interaction of a substance M present particularly in *Nicotiana Rusbyi* and *Datura Wrightii* with a substance N present in many of the other *Solanaceae*.

TABLE XXIX.

EFFECT OF HEAT ON THE REACTIVE SUBSTANCES RESPONSIBLE FOR THE AB REACTION.

A	B	Precipitin reaction of A with B;							
Normal extract:	Heated extract:	Unheated control(B)	B heated to: 40°	50°	60°	70°	80°	90°	100°
Platanus	N.Rusbyi	1	1	1	1	1	1	1	1
Ribes	N.Rusbyi	2	2	2	2	2	2	2	2
Platanus	N.Tabacum	1	1	1	1	1	1	1	1
Ribes	N.Tabacum	1	1	1	1	1	1	1	1
Platanus	N.rustica	2	2	2	2	2	2	2	2
Ribes	N.rustica	2	2	2	2	2	2	2	2
Platanus	Dat.Wright.	2	2	2	2	2	2	2	2
Ribes	Dat.Wright.	2	2	2	2	2	2	2	2

The first test regarding the nature of the AB reaction was with regard to heat. Fresh leaves of *Nicotiana Rusbyi* (B), *N. rustica* (B), and *Datura Wrightii* (B) were extracted for 18 hours in physiological saline. They were then filtered and the normal extracts were used in the following tests. Normal aqueous extracts of dried leaves of the following were also used: *N. Tabacum* (B), *Platanus* (A), and *Ribes* (A). The solanaceous extracts were then divided each into 8 fractions and the various fractions were heated in water baths for ½ hour intervals at temperatures ranging from 40°C. to boiling. The results of this experiment are given in Table XXIX.

Several considerations develop from a study of this table.

1. The B factor (but not necessarily the A factor as far as we know at this point) is not rendered inactive by heating for ½ hour at temperatures up to 100°C. There was observed in some cases a very slight drop in potency in the extracts heated at temperatures above 58°C., but this was so slight as not to be scoreable. In any case

there was still a reaction approximately equal to the control reaction in all the various heated extracts. There was a heavy precipitation due to heating in all the extracts but *N. Tabacum,* but removal of this precipitate did not influence the reaction.

2. The extracts of *N. Rusbyi* and *D. Wrightii* were tested against *N. Tabacum* and *N. rustica* at the beginning of this experiment, but the results (in contrast with those of Table XXVII) were negative or very weak. Thus the MN reaction is dependent either upon the variability of different plants or on the solvent used in extraction. This applies to both *N. Rusbyi* and *D. Wrightii.* Many subsequent extractions have confirmed this finding: There is considerable variation with regard to the presence and strength of the M principle in experimental plants of the same species. This fact, however, does not apply to the other principles studied thus far.
3. The AB reaction is manifestly different from the MN reaction, in that extracts wholly negative for the one are strongly positive for the other. Both are distinct from the calcium oxalate reaction, since in this experiment the tests with $Ca(NO_3)_2$ remove the possibility that a calcium oxalate reaction could be involved here.

As a further test of the effect of heat on these reactions, normal extracts of *Ribes, Platanus, N. Rusbyi,* and *N. Langsdorffii* were prepared. In this case the *N. Rusbyi* (M) was negative to the *N. Langsdorffii* (N). Each extract was divided into two samples, one sample being retained as a control, the other heated in flowing steam at 0-2 lbs. pressure for 3 hours. The *Ribes* and *Platanus* extracts remained clear throughout the heating, the other two species autoprecipitated and were cleared before testing. The results of the precipitin tests, which were then performed, are given in Table XXX.

It will be seen from this experiment that the AB reaction is unaffected by heating the extracts for 3 hours at 100°C. This applies to both A and B principles.

Further tests of the AB and MN reaction were performed under similar conditions. Active A, B, M, and N extracts were prepared in the customary way, heated and cleared as in the preceding experiment, and tested as in Table XXXI.

Here the results confirm those of the preceding experiment in showing the AB reaction to be coctostable, while there is a weakening of both M and N principles due to the heating. In this respect, then, as well as in others which will be seen later, the AB reaction differentiates itself from the MN reaction. Tests with potassium oxalate demonstrated that the calcium oxalate reaction was not involved here.

TABLE XXX.

EFFECT OF LONG-CONTINUED HEATING ON THE AB REACTION.

		Precipitin reaction with:			
		Nicotiana Rusbyi		N.Langsdorfii	
		Control	Heated	Control	Heated
Ribes.....Control..		2	2	2	2
Ribes.....Heated...		2	2	2	2
Platanus..Control..		1	1	1	1
Platanus..Heated...		1	1	1	1

TABLE XXXI.

EFFECT OF LONG-CONTINUED HEATING ON THE AB AND MN REACTIONS.

	Precipitin reaction with:			
	Ribes		N.Rusbyi	
	Control	Heated	Control	Heated
N.alata...Control	1	1	3	2
N.alata...Heated	1	1	2	1

TABLE XXXII.

APPLICATION OF SILBERSCHMIDT'S METHODS OF PRE-EXTRACTION AND SOLUTION TO THE AB AND MN REACTIONS.

		No pre-extraction:					Pre-ex.in alc.			Pre-ex.in alc.eth.chl.			
Normal extract:	Experim. extract:	H_2O	NaCl	H_2O+ NaCl	NaCl +MgO	NaOH	H_2O	NaCl	NaCl +MgO	H_2O	H_2O+ NaCl	NaCl	NaCl +MgO
(MN reaction)													
N.Rustica	N.Rusbyi	2	2	2	2	(1)	0	0	0	0	0	0	0
N.Rusbyi	N.rustica	2	2	2	2	(1)	1	1	t	t	t?	t	t
(AB reaction)													
N.Rusbyi	Platanus	2	2	2	0	0	1	1	0	t	t	t	0
N.Rusbyi	Ribes	3	3	3	1	(t?)	2	2	0	1	1	2	0
N.rustica	Platanus	1	1	1	0	(1)	t	t	0	t	t	t	0
N.rustica	Ribes	2	2	2	2	(1)	1	1	t	1	t	1	t
Platanus	N.Rusbyi	2	2	2	2	(1)	t	t	1	t	t	t	1
Platanus	N.rustica	2	2	2	2	(2)	t	t	1	t	t	1	1
Ribes	N.Rusbyi	3	3	3	3	(1)	1	2	2	t	2	2	2
Ribes	N.rustica	3	3	3	3	(3)	2	1	2	t	1	1	t

B. Experiments in Extraction and Solution

As an extension of the scope of the experiment reported on page 149 regarding the effect of Silberschmidt's various techniques upon the resultant reactions, this experiment was repeated with the AB and MN reactions. The plan of the experiment was exactly as in the earlier one. Pre-extractions were of three types: (a) none, (b) with 95% alcohol + tartaric acid, and (c) with tartaric alcohol followed by ether, chloroform, and chloroform vapor. Solution was in water, physiological saline, water + solid NaCl in concentration of .85%, physiological NaCl + MgO to neutrality, and N/20 NaOH. The results of this experiment are given in Table XXXII.

The reactions involving NaOH are not considered significant because of the reaction of the controls with a pure solution of this solvent. These reactions thus represent alkalinity coagulations and not true precipitin reactions. The remainder of the reactions are all significant, however.

With regard to principle M, this experiment shows that it is completely soluble or denatured in 95% alcohol under these experimental conditions. N is somewhat affected by 95% alcohol. The use of physiological saline in place of water as the solvent exerts no perceptible effect on the reaction, regardless of whether the NaCl is present after or before extraction. Extracts in water may thus be freely tested against extracts in NaCl without artefact reactions appearing (this point has also been many times confirmed in subsequent tests).

Principle A is moderately soluble or denatured in alcohol and the lipoid solvents. It is soluble preferably in acid solution, not in neutral solution, and it is equally soluble and reactive in water and physiological saline. Principle B, on the other hand, is equally soluble in water and in physiological saline and in acid and neutral solutions; it is soluble or denatured to a moderate extent in alcohol and the lipoid solvents.

Thus this experiment establishes definitely the fact that the AB and MN reactions are not only distinct from the calcium oxalate reaction (which was eliminated in this experiment by the control tests) but that they are distinct from each other. That the A and B principles are definitely soluble or denatureable in alcohol points to their organic nature. This experiment likewise indicates that the techniques used by Silberschmidt may exert a very distinct effect on the strength of the reactions observed. Some of the inconsistencies which he was unable to account for undoubtedly are to be explained in terms of the solubilities of the reactive substances, and a subsequent part of this paper will

accordingly be devoted to a consideration of his results in the light of this and other experiments.

The question of alcohol solubility thus appears to be a very important one, and accordingly a second, more thorough experiment concentrating on this factor was devised. Leaves of *Platanus* (A), *Ribes* (A), *Nicotiana tomentosa* (B, N), and *N. Rusbyi* (M) were dried and pulverized; 1 gm. of each was extracted in physiological saline for 2 hrs. at room temperature. One gm. of each was pre-extracted in *many changes of absolute alcohol;* 1 gm. of each was pre-extracted in many changes of anhydrous ether. There were about 9 changes of pre-extractant in each case, each change lasting 2 hours. The pre-extracted supernatants were evaporated down and redissolved in physiological saline for testing. The pre-extracted residues were dried and extracted in physiological saline. Thus for each species the following 5 extracts were available:

a. No pre-extraction, whole extract (control) in physiological saline.
b. Alcohol extractive in physiological saline.
c. Alcohol residue in physiological saline.
d. Ether extractive in physiological saline.
e. Ether residue in physiological saline.

These were tested against each other according to the scheme of Table XXXIII.

TABLE XXXIII.

EFFECT OF THOROUGH PRE-EXTRACTION WITH ALCOHOL AND ETHER ON THE AB AND MN REACTIONS.

Effect on principle M:

Rusbyi	a +	tomentosa	a...2		Ribes	a +	tomentosa	a...1		Platanus	a +	tomentosa	a...1
"	b +	"	a...0		"	b +	"	a...1		"	b +	"	a...1
"	c +	"	a...2		"	c +	"	a...1		"	c +	"	a...1
"	d +	"	a...0		"	d +	"	a...0		"	d +	"	a...0
"	e +	"	a...2		"	e +	"	a...t		"	e +	"	a...t

Effect on principle N:

Rusbyi	a +	Tomentosa	a...2		Ribes	a +	tomentosa	a...1		Platanus	a +	tomentosa	a...1
"	a +	"	b...0		"	a +	"	b...t		"	a +	"	b...t
"	a +	"	c...2		"	a +	"	c...t		"	a +	"	c...t
"	a +	"	d...0		"	a +	"	d...0		"	a +	"	d...0
"	a +	"	e...2		"	a +	"	e...1		"	a +	"	e...1

Effect on M and N:

Rusbyi	a +	tomentosa	a...2		Ribes	a +	tomentosa	a...1		Platanus	a +	tomentosa	a...1
"	b +	"	b...0		"	b +	"	b...1		"	b +	"	b...t
"	c +	"	c...2		"	c +	"	c...1		"	c +	"	c...t
"	d +	"	d...0		"	d +	"	d...0		"	d +	"	d...0
"	e +	"	e...1		"	e +	"	e...1		"	e +	"	e...t

Effect on principle B:

Ribes	a +	tomentosa	a...1		Platanus	a +	tomentosa	a...1					
"	a +	"	b...t		"	a +	"	b...t					
"	a +	"	c...t		"	a +	"	c...t					
"	a +	"	d...0		"	a +	"	d...0					
"	a +	"	e...1		"	a +	"	e...1					

Effect on principles A and B simultaneously:

Ribes	a +	tomentosa	a...1		Platanus	a +	tomentosa	a...1					
"	b +	"	b...1		"	b +	"	b...t					
"	c +	"	c...1		"	c +	"	c...t					
"	d +	"	d...0		"	d +	"	d...0					
"	e +	"	e...1		"	e +	"	e...t					

From a consideration of this table one may conclude that under these more thorough experimental conditions:

1. Principles A, B, M, and N are all insoluble in ether and unaltered by ether treatment. They are hence non-lipoid.
2. Principles A, B, M, and N are all non-coagulable by alcohol, even when treated for 24 hours with absolute alcohol at 50°C.
3. *Principles M and N are wholly insoluble in absolute alcohol.*
4. *Principles A and B are both very appreciably soluble in absolute alcohol.*
5. No difference was seen in the behavior of A from that of B nor in M from that of N.

The results of these two experiments are summarized in the following table for the reader's convenience (Table XXXIV).

TABLE XXXIV.

SOLUBILITY AND REACTIVITY OF PRINCIPLES A, B, M, AND N IN VARIOUS SOLVENTS.

Reactive principle:	Solubility in: Alcohol	Ether	Reactivity in: Water	Water + NaCl	.85% NaCl	NaCl + MgO	NaOH
calcium oxalate	None	None	Good	Good	Good	Good	Results not signif-icant
"A"	Moderate	None	Good	Good	Good	Poor	because of
"B"	Moderate	None	Good	Good	Good	Good	artefact.
"M"	None	None	Good	Good	Good	Good	.
"N"	None	None	Good	Good	Good	Good	.

C. Experiments in Dialysis

Dialyses of the extracts to determine the diffusability of the AB and MN principles were performed in the same fashion as with *Platanus*, *Prunus*, and *Robinia* as described on page 135. No further comment is necessary beyond the statement that the dialyses of the *Solanaceae* were usually performed using physiological saline rather than distilled water as the fluid outside the membrane. This was desirable practically because it is difficult to obtain clear extracts of *Nicotiana Rusbyi* and certain other *Solanaceae* in water, and theoretically because the protein which might hypothetically be responsible for these reactions might be precipitated by removal of the electrolytes. The experiments of the previous section, however, have revealed that there is no choice in the use of these two solvents as far as demonstrating the precipitin tests is concerned, and furthermore in all experiments here and elsewhere where both aqueous and saline extracts were used in the same experiment,

control tests against the respective solvents confirmed the absence of artefact reactions due to choice of solvent. Since the $AgNO_3$ test for freedom from chloride could not be used in dialyses in NaCl, the oxalate-calcium test, of comparable sensitivity, was substituted where necessary. The results of the dialysis experiments are gathered in Table XXXV.

Considering first the dialyses of *Platanus* (A) one observes that the

TABLE XXXV.

EFFECT OF DIALYSIS ON THE REACTIVE PRINCIPLES OF REACTIONS AB AND MN.

A. Effect on principle A

Extract dialyzed:	Nature of extract:	Exp. #:	Dialysis tests: Prot.	Cl.	Ca.	Sugar	Precipitin tests of experimental extract against: Extract:	Test:	Extract:	Test:	Extract:	Test:
Platanus	Normal	5-10	4	4	3		N.tomentosa	3	N.glauca	3		
"	Dialyz.	8	2	t	t		"	2	"	1		
"	"	9	3	t	t		"	2	"	2		
"	"	7	2	2	t		"	1	"	2		
"	"	5	2	t	t		"	1	"	2		
"	"	10	3	1	t		"	2	"	1		
"	Diffus.	8	0	4	1		"	0	"	0		
"	"	9	t	4	3		"	0	"	0		
"	"	7	0	4	3		"	0	"	0		
"	"	5	0	4	3		"	0	"	0		
Robinia	Normal	19-26	4	4	4		"	3	"	3		
"	Dialyz.	24	3	1	t		"	3	"	2		
"	"	20	2	1	t		"	2	"	2		
"	"	25	4	2	t		"	3	"	3		
"	"	23	4	1	t		"	2	"	2		
"	"	22	2	1	t		"	2	"	2		
"	"	26	4	2	1		"	2	"	3		
"	"	21	4	2	0		"	2	"	3		
"	"	19	3	1	t		"	3	"	2		
"	Diffus.	19	2	4	2		"	2	"	1		
"	"	21	1	4	2		"	1	"	2		
"	"	22	2	4	2		"	1	"	2		
"	"	23	2	4	2		"	2	"	2		
"	"	20	4	3	1		"	2	"	3		
"	"	24	2	4	2		"	2	"	3		
"	"	25	1	4	2		"	1	"	2		

B. Effect on principle B

N.tabacum	Normal	27	3	4	3				Platanus	1		
N.tomentosa	"	34	4		3	4	Robinia	2	"	1		
"	"	35	4		4	4	"	2	"	1		
N.rustica	"	37	2		4	4			"	2	Ribes	4
N.alata	"	38	2		4	3	"	2	"	1	"	2
"	"	39	2		3	3	"	2	"	1	"	2
"	"	40	4		3	2	"	2	"	1	"	2
"	"	41	4		4	2	"	2	"	1	"	2
"	"	42	2		4	4					"	1
"	"	43	2		4	4					"	1
"	"	44	2		3	4					"	2
"	"	45	2		3	4					"	2
N.tabacum	Dialyz.	27	2	1					"	t		
N.tomentosa	"	34	3		t	0	"	1	"	1		
"	"	35	3		t	0	"	1	"	1		
N.rustica	"	37	1		0	0			"	1	"	2

TABLE XXXV. (Continued).

Extract dialyzed:	Nature of extract:	Exp. #	Dialysis tests: Prot.	Cl.	Ca.	Sugar	Precipitin tests of experimental extract against: Extract:	Test:	Extract:	Test:	Extract:	Test:
N.alata	Dialyz.	38	1		t	0	Robinia	1	Platanus	0	Ribes	1
"	"	39	1		t	0	"	1	"	0	"	1
"	"	40	2		0	0	"	1	"	0	"	1
"	"	41	2		0	0	"	1	"	0	"	1
"	"	42	2		0	t					"	1
"	"	43	2		0	t					"	1
"	"	44	1		t	t					"	2
"	"	45	1		t	t					"	2
N.tabacum	Diffus.	27	0	4			"	1				
N.alata	"	44	t		3						"	0
"	"	45	t		3						"	0
C. Effect on principle M												
N.Rusbyi	Normal	28	4		4				N.tabacum	1	N.rustica	3
"	"	29	4		4				"	1	"	3
"	"	33	3		3	4	N.nudicaulis	1			"	2
"	"	34	3		3	4	"	1			"	2
"	Dialyz.	28	4		0				"	1	"	2
"	"	29	4		0				"	1	"	2
"	"	33	3		0	0	"	1			"	2
"	"	34	3		0	0	"	1			"	2
D. Effect on principle N.												
N.alata	Normal	38	2		4	3	N.Rusbyi	2				
"	"	39	2		4	3	"	2				
"	"	40	4		4	2	"	3				
"	"	41	4		4	2	"	3				
"	"	42	2		4	4	"	3				
"	"	43	2		4	4	"	3				
"	"	44	2		3	4	"	2				
"	"	45	2		3	4	"	2				
"	Dialyz.	38	1		t	0	"	0				
"	"	39	2		t	0	"	0				
"	"	40	2		0	0	"	0				
"	"	41	2		0	0	"	0				
"	"	42	2		0	t	"	0				
"	"	43	2		0	t	"	0				
"	"	44	2		t	t	"	0				
"	"	45	2		t	t	"	0				
"	Diffus.	44	t		3		"	2				
"	"	45	t		3		"	1				

Notes: Prot.: Millon test for ptotein; Cl.: $AgNO_3$ test for chloride; Ca.: potassium oxalate test for calcium; Sugar: Fehling test. Extracts in right hand columns all normal. Dialyz.: Dialyzate; Diffus.: Diffusate.

technique of dialysis was satisfactory as is evinced by the dialysis tests. The fractionation resulted in a retention of most of the Millon protein and very little of the crystalloid content. The reactive principle A was retained in amount comparable to the retained protein in the dialyzates. The diffusates, protein-free and containing almost all of the crystalloids, were absolutely negative to the precipitin tests. The results with *Robinia* are less clear cut, but still significant. The dialyzates contained the bulk of the protein and an appreciable but minor amount of crystalloids. The bulk of the salts had plainly passed through to the diffusates, although some protein had also escaped. The results may be

considered safe by comparison, however, the precipitin reaction as in *Platanus* being strongest in the high-protein, low-crystalloid fraction. We may thus conclude from these dialyses that *principle A diffuses very slowly through membranes relatively impermeable to protein.*

Turning now to principle B, the dialyses were technically highly efficient. Most of the protein was reclaimable in the dialyzates, but the latter were practically or entirely negative for calcium and sugar. However, with this highly efficient fractionation, nevertheless a very appreciable amount of the precipitin reaction was lost in dialysis. This is true of the various species employed in these B dialyses all of which behaved similarly. It is thus apparent that, like principle A, *principle B diffuses, although imperfectly and slowly, through membranes highly impermeable to protein but highly permeable to crystalloids.*

The results with the M and N principles are beautifully clear-cut and striking. With regard to principle M in *Nicotiana Rusbyi* the dialyzates retained *all* the protein and *none* of the calcium or sugar according to the dialysis tests. Moreover the precipitin reaction was retained in its full strength in the dialyzates. On the other hand, although the dialyses were almost equally sharp and clear-cut with regard to principle N, the latter was completely lost from the dialyzates in company with the crystalloid content, although very little protein passed through the membranes. We may hence conclude that *principle M is retained by protein-impermeable membranes with a high degree of efficiency, while principle N readily passes through such membranes.*

D. Nature of the AB, MN, and XY Reactions

The experiments in dialysis conclude our investigation of the nature of the AB and MN reactions as far as it has been accomplished at the present time. However, although it is impossible at the present definitely to ascribe these reactions to specific substances, yet from the data obtained one can eliminate many possibilities and arrive at an approximate idea of the nature of the reactive substances involved. We are now able to utilize the following facts which have been demonstrated above.

Principles A and B are water soluble, alcohol soluble, coctostable, insoluble in lipoid solvents, not coagulated by alcohol, and slowly and imperfectly dialyzable through membranes relatively impermeable to protein. The precipitate resulting from their interaction chars markedly on ignition. It is thus plain that they are organic, non-lipoid, and non-protein (because of their alcohol solubility and positive, although imperfect, dialysis). The only alcohol-soluble proteins, the prolamines,

are insoluble in water (19). Thus far no satisfactory distinction has been found between A and B.

Principles M and N are likewise soluble in water and weak salt solutions, but are insoluble in alcohol, non-coagulable by alcohol, insoluble in lipoid solvents, and in contrast to A and B are thermolabile. The precipitate formed by their interaction chars on ignition. M is retained by dialyzing membranes with high efficiency, but N passes freely through such membranes. Thus we may conclude that M and N are both organic and non-lipoid. N is in all probability an organic substance of relatively simple structure, and in any case it is highly unlikely that it is protein. M, on the other hand, gives strong indications of being a protein or at least a complex organic substance of high molecular weight.

With regard to the XY reaction, nothing can be said as to its nature at the present. This is the least frequent, and because of its weakness and rarity the least significant of the four reactions studied. It is plain from Table XXVIII that the species of *Datura, Physalis,* and *Atropa* are alike in possessing a component X which reacts with *Ligustrum* (Y). Whether or not the reactions of *Solanum Capsicastrum* against the *Oleaceae* are qualitatively the same or different is questionable. Because of the weakness and infrequency of this reaction or group o reactions, no analytical tests have been employed concerning it.

VII. THEORETICAL AND PRACTICAL SIGNIFICANCE OF THIS STUDY

In the light of the investigations reported in this paper, it is now of importance briefly to review the results obtained by the various investigators in the field of the plant precipitin reactions for the purposes of culling out the interpretations now known to be erroneous, of supporting with experimental proof those which are correct, of evaluating the immunological significance of the plant precipitin reactions, and of thus placing the studies regarding such reactions upon a solid experimentally-determined foundation. Accordingly the present section will be devoted to these more important theoretical and practical considerations.

A. Analysis of Kostoff's Results in the Light of the Present Study

Kostoff's studies on the precipitin reaction in plants (12) were restricted to 20 species and a hybrid of the *Solanaceae*. The materials on which the findings of the present study are based included 16 of the 20 species on which Kostoff worked. Of the 157 *normal* precipitin reactions of these 20 species tabulated by Kostoff we have repeated all

but 52, with the following results (compiled from Kostoff's Table 10, l. c. p. 49, and from our Tables XXV and XXVI):

Negative reactions reported by Kostoff and confirmed by us 64
Positive reactions reported by Kostoff and confirmed by us 33
Due to calcium oxalate 23
Due to the MN reaction 10
Reactions (positive and negative) reported by Kostoff and not confirmed by us 53
Positive reactions reported by Kostoff and found by us to be negative 7

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Note: With regard to *Capsicum*, Kostoff used *C. pyramidale* while we used *C. frutescens*, but the reactions of these two species are clearly the same, judging from his and our data. The other species involved were identical and largely from the same stock.

Of the *27 acquired* precipitin reactions described by Kostoff in the *Solanaceae* we have repeated the normal reactions in 10 of the combinations with the following findings:

Marked acquirement of precipitins according to Kostoff 5
Found by us to be due to calcium oxalate 3
Found by us to be due to the MN reaction 1
Not tested by us 1
Slight acquirement of precipitins according to Kostoff 7
Found by us to be due to calcium oxalate 0
Found by us to be due to the MN reaction 2
Found by us to be negative 1
Not tested by us 4
No acquirement of precipitins according to Kostoff 11
Found by us to be due to calcium oxalate 1
Found by us to be negative 9
Not tested by us 1
Decrease of precipitins according to Kostoff 4
Found by us to be due to the MN reaction 3
Not tested by us 1

We thus see that of the 33 normal precipitin reactions reported by Kostoff and repeated by us, 70% were due to calcium oxalate and 30% to the MN reaction. It is of interest to compare these results with those obtained by us in all our normal precipitin tests. The total number of combinations tested by us was 869. Of these, 444 were negative and 425 positive. Of the 425 positive tests 242, or 57%,

were due to the calcium oxalate reaction, 100, or 24%, were due to the AB combination, 73, or 17% were due to the MN reaction, and 10, or 2+% were due to the XY pair.

Thus it is evident that in so far as they have been repeated, Kostoff's positive reactions were all due to the calcium oxalate and MN reactions, mainly to the former. Since the calcium oxalate reaction is inorganic and more or less incidental to the autonomy of the plant, it is manifest that no immunological conclusions can be drawn from an observation of such reactions. Moreover, with regard to the MN reaction, since one reactive group (N) at least is plainly non-protein, and since in the majority of cases where Kostoff dealt with this reaction he observed no change or even decrease, rather than increase, in precipitin reaction after grafting, no sound immunological conclusions may be drawn from this reaction. Kostoff's experiments in nephelometric determination of precipitins (13, 14, 15, 15a) are subject to the same criticism as his gross precipitin tests.

Kostoff's experiments *in vitro* include a discussion of "lytic rings" at the line of junction of the two extracts. These, he believes, are comparable to lytic reactions in animals. In later papers (14, 15, 15a) Kostoff has reported a confirmation of the reactions believed by him to be lytic, by means of dialysis-Ninhydrin tests. Regarding these last tests the authors have nothing to say, since they have not yet been repeated. But with regard to Kostoff's "lytic rings" these have been frequently observed and studied. In his first paper on this subject (3) the senior writer mentioned having observed them in his oleaceous tests but he found them to be "not consistent, weak, and apparently of no greater significance." He was unable, however, to demonstrate lysins in the oleaceous extracts in preliminary experiments using the Ninhydrin method.

That the "lytic ring" phenomenon is not immunological in nature is evident from the following observation transcribed from our notes on one of the preceding experiments. "On May 4th as a control of Experiment 83 the *Ribes Carrierei* extract (N/2 in physiological saline) was layered with N/100 potassium oxalate. The ring resulting (calcium oxalate) was about +1 in strength, clear, and showed the clearest, most striking double ring separated by a clear zone which I have ever seen" (Chester). This was confirmed by two other observers, and was undoubtedly Kostoff's "lytic ring," but occurring in the layering of an extract with an inorganic salt! The same was repeated several times the following day with similar results in all cases. It was not a function of any particular way of pipetting, but careful layering was necessary to bring it out.

It is therefore evident that Kostoff's precipitin reactions and his "lytic rings" are both of the same types as the corresponding phenomena studied by the writers, which are assuredly non-immunological in nature, and that accordingly, on the basis of the data published thus far by Kostoff, one is not justified in drawing immunological conclusions.

B. Analysis of Silberschmidt's Results in the Light of the Present Study[1]

Silberschmidt's experiments (21) dealt with the following species of *Solanaceae*: *Nicotiana Tabacum, N. rustica, N. glutinosa, Solanum Dulcamara, Lycium barbarum*, and *Salpiglossis sinuata*. According to our findings these species had the following complements of reactive principles. The three species of *Nicotiana* all contained an excess of calcium and of principle N; *Salpiglossis* contained excess oxalate and lacked both M and N. From the behavior of Silberschmidt's other two species, *Solanum Dulcamara* probably contained a weak excess of calcium and principle N, while *Lycium* probably contained a slight excess of oxalate and lacked M. The calcium ion was evidently strongest in Silberschmidt's *N. Tabacum* and *N. rustica* and rather weak in *S. Dulcamara* and *N. glutinosa*, while the oxalate was evidently stronger in *Salpiglossis* than in *Lycium*, but not very strong in either.

Calcium oxalate reactions are plainly evident from Silberschmidt's tables (e.g. *Salpiglossis* + *N. rustica* and *N. Tabacum* in his Table 2). The clearness of his readings was at times obscured by the cloudiness and dilution of his extracts. This applies particularly to his data in Tables 3, 5, and 6. In his Tables 9, 10, 11, and 12 the reactions are all negative or practically so as would be expected from such reactive complements as those outlined above. The only reactions requiring comment are two involving *N. Tabacum*. In Silberschmidt's Tables 3 and 4 *N. Tabacum* reacts positively with *Solanum Dulcamara* and with *N. rustica*. This *N. Tabacum* reaction behaves much as the MN, and it would be reasonably explained on the assumption that *N. Tabacum* (which is closely related to *N. Rusbyi*, the type of principle M), sometimes contains a sufficient quantity of M to react with active N extracts. In accordance with this view is the fact that Kostoff's *N. Tabacum* reacted, although weakly, with two of the other *Nicotiana* species used. As was mentioned earlier in this paper the presence of M in *N. Rusbyi* varies considerably from plant to plant and a corresponding variability

[1] A second paper by this author has recently been published. This paper [Silberschmidt, K. (1932). Studien zum Nachweis von Antikörpern in Pflanzen. II. (Planta: Arch. f. Wiss. Bot. 17:493-589)] is subject to the same criticism as the paper under discussion.

in *N. Tabacum* would perfectly account for the apparent discrepancies between Silberschmidt's results and those of Kostoff and the present writers.

Such an analysis accounts for such of Silberschmidt's reactions as are significant, while his negative results in the few places where one would expect positive reactions are apparently due either to lack or to weakness of reactive principles in the various combinations. His one acquired reaction is hardly of strength to be considered significant.

Hence one may conclude, with regard to Silberschmidt's work, that it is confirmed in fact, but not in theory, by the present study. For the same criticisms regarding immunological interpretations as were made of Kostoff's results are applicable to those of Silberschmidt. His positive reactions are all explainable in the terms of the relatively simple reactions described in this paper, and there is no evidence that any of them are due to protein interaction.

C. Analysis of East's Results in the Light of the Present Study

East (7) has observed certain phenomena *in vivo* with regard to recovery of sugar cane from the mosaic disease and to temporary resistance to re-infection. In connection with these observations he carried out a series of precipitin tests in order to try to decide whether or not the proteins of various types of cane were different. His experimentation followed the same general type as that used by Kostoff and Chester, and accordingly his results may be considered comparable. In certain combinations of extracts of cane with those of cane and other plants East obtained positive results. He found some indication of a consistent difference in precipitin reaction between cane which had never had the mosaic disease and cane which had or may have had the disease. Since plants which exhibited the mosaic disease reacted in the same manner as plants which appeared free from disease but which might have been acting as carriers, and since the reactions of such plants consistently differed from those of cane known never to have had the disease, East believed that his results, including his observations on infection and recovery, pointed to the probability that sugar cane gains an apparent immunity by reducing the virulence of the mosaic virus.

The results of the present study render it very questionable as to whether the precipitin experiments with sugar cane should be considered immunological. Since the writers have not experimented with the species of plants used by East, it is impossible dogmatically to relegate his reactions to the same category as the non-protein reactions found to occur so extensively in the *Solanaceae* et al., but assuredly one

must be rather skeptical in accepting as immunological any precipitin experiments in which the possibilities of such non-protein reactions as the calcium oxalate and AB reactions are not eliminated.

D. Analysis of Chester's Results in the Light of the Present Study

The findings of Chester with regard to the precipitin reaction (3, 4, 5) have not been definitely interpreted as immunological, pending the results of the present study. Chester's findings prior to this study may be stated in the following terms:

1. No normal precipitins were found in the *Oleaceae* studied.
2. Graft-blighted Lilacs showing serious malnutritional symptoms gave strong positive tests with other *Oleaceae* regardless of systematic relationship to stock or scion. Lilacs suffering from similar symptoms but ungrafted gave the same reactions.
3. In tests in a number of families of woody plants a clear-cut parallel was observed between the precipitin reaction and the systematic relationships of the plants tested.

It is the purpose of the present section to criticize and interpret these findings.

The tests reported in this paper have shown that in every case tested, the *Oleaceae* were characterized by having an excess of calcium present in their extracts. In the morbid processes attending the slow dying of leaves of Lilac there is an accumulation of oxalate. This has been shown by the fact that reactive extracts of graft-blighted plants may be rendered inactive toward "calcium" extracts by precipitating them with a slight excess of calcium. Such oxalate accumulation is localized in the diseased and dying cells, since when the green portions of green-and-yellow mottled leaves are cut away from the yellow portions and extracted, the extracts of these green portions behave exactly like extracts of healthy Lilacs. This fact renders it still more improbable that the reactions of graft-blighted Lilacs are immunological, since hypothetical antibodies from grafting would be expected to be generally distributed through the leaf tissue. The fact that leaves of plants mechanically injured are also reactive in the yellow areas supports this simple interpretation, as does the fact that extracts of mottled leaves autoprecipitate in extraction, losing their reactive potency as auto-precipitation continues, due to the interaction of the excess oxalate in the yellow portions with the excess calcium in the green portions.

Passing next to the highly absorbing topic of the specificity of the normal precipitin reactions thus far investigated, the results of this

study are of especial interest. *A priori* it was difficult to conceive that such specificity as was seen in the *Prunoideae* and the other groups of woody plants studied could be due merely to the interaction of relatively non-specific substances. However the distribution of many chemical substances in plants follows in general their relationships. Thus the presence and form of calcium oxalate crystals in plants have been frequently used as taxonomic criteria, and it is manifest from a study of the data on distribution of other substances, such as saponins, glucosides, alkaloids, etc., that homogeneous groups of plants are characterized by homogeneity in their content of such substances.

Guided by such reasoning it was of interest to examine the distribution of the various reactive principles differentiated in this study to observe whether they showed any species-, genus-, or family-specificity in their distribution among the various plants studied. From the results of Tables XXV-XXVIII it was possible to ascribe to each species its "reactive formula." This, for example, with *Salpiglossis sinuata* was: A—B+, M—N—, X—Y—, Ox+Ca—. These were formulated for each of the species studied and then they were grouped in a trifurcating dendritic system as in Figure IV. The order of arrangement was guided by the observation that certain of the reactions varied within the genus, others within the family, etc.

A survey of Figure IV brings out some very interesting relationships. The five families used in this study fall into three orders, the *Rosiflorae*, including the closely related *Platanaceae*, *Saxifragaceae*, and *Leguminosae* (which three are adjacent in Rehder's Manual), the *Contortae*, including the *Oleaceae*, and the *Tubiflorae*, represented by the *Solanaceae*. With but one questionable exception the *Rosiflorae* studied all possess A and lack B, the *Tubiflorae* (*Solanaceae*) all contain B and lack A, while the *Contortae* (*Oleaceae*) all lack both A and B. *Prunus* lies very near the borderline, and might without difficulty be included in either group. Within the *Rosiflorae* subsequent division was not attempted because of the difficulty of eliminating the AB reaction in considering the MN and XY reactions. Within the *Oleaceae* all contain excess of Y and of calcium and are separated only by presence or absence of M and N, a highly homogeneous assortment of reactive principles as would be expected from their relative taxonomic homogeneity.

Within the *Solanaceae* none contain the principle Y (which was found in the *Oleaceae*) and the majority also lack X, although this is present in a few species. The *Solanaceae* are differentiated by both the MN and calcium oxalate reactions, the latter, however, being con-

sidered, because of its simple nature, as least significant. Of the 18 species of *Nicotiana* all are identical in their reactive ingredients with the sole exception of *N. Rusbyi*. *Datura* varies with regard to the XY reaction but is relatively uniform with regard to the presence of N and

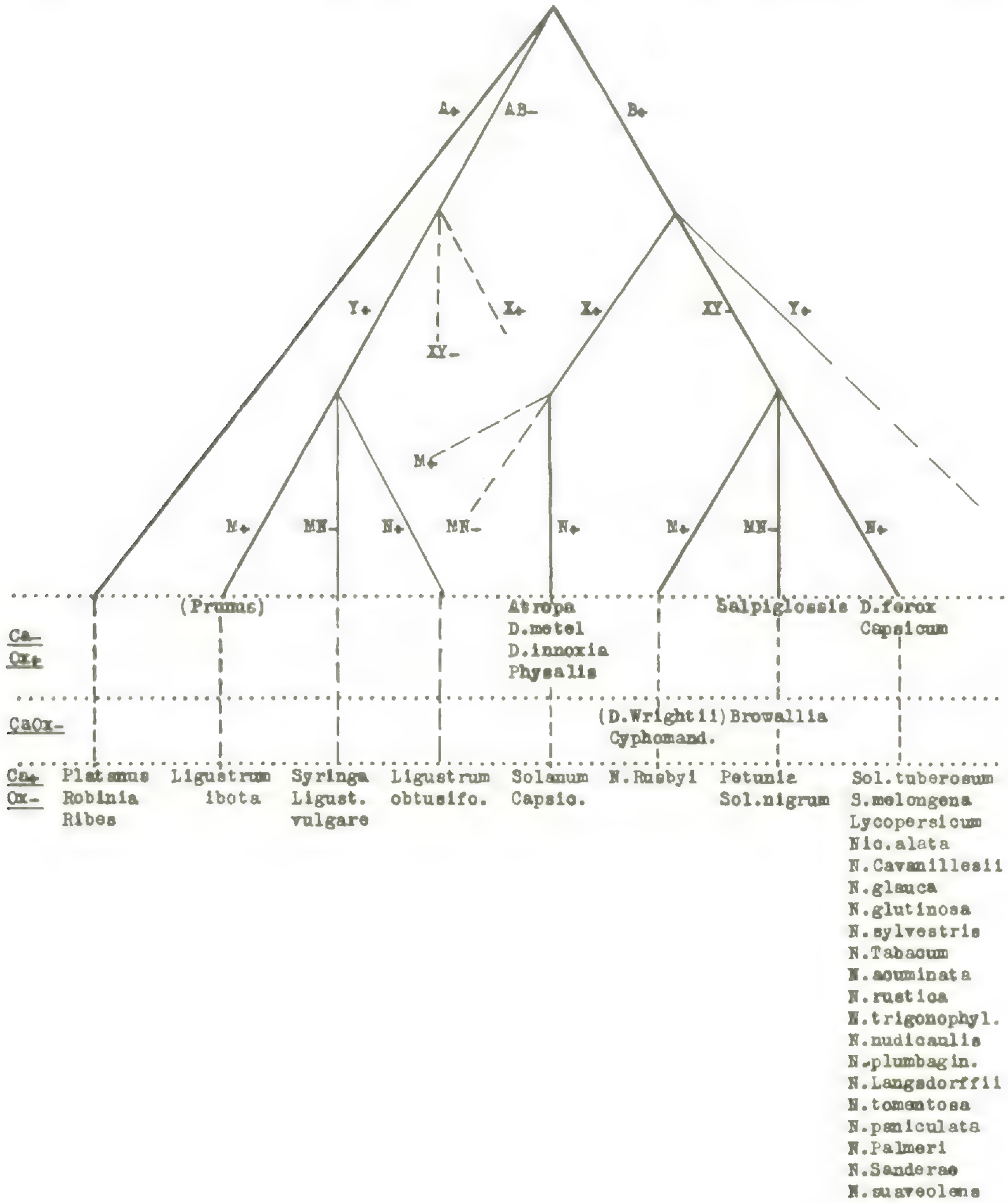

FIGURE IV. CLASSIFICATION OF ALL EXTRACTS STUDIED ACCORDING TO THE PRESENCE OR ABSENCE OF THE VARIOUS REACTIVE PRINCIPLES.

of excess oxalate. The *Solanum* species are all uniform in their excess of calcium, although there was some individual variation of this in *S. tuberosum*, while they are separated by the presence or absence of MN and X.

Taken on the whole, there seems to be a definite tendency for the various taxonomic units to show relatively homogeneous reactive formulae. There are exceptions, to be sure, but these exceptions are by no means as striking as the agreements. That such a dendritic system as that of Figure IV could result from chance is beyond belief. It is entirely possible that an extension of these results to include many more species might either favorably or unfavorably influence this relationship, but the data presented appear to justify, at the present, the statement that there is a definite indication of a correlation between the distribution of reactive principles in the plants studied and their taxonomic position.

Hence the results obtained by the senior writer on the *Rosaceae, Saxifragaceae, et al.* are interpreted by the results of the present study. If there is even a moderate and imperfect degree of correlation between the systematics of these plants and their complement of reactive principles, then it necessarily follows that this correlation will be reflected in a corresponding correlation of precipitin reaction with systematics.

Before, however, drawing final conclusions regarding the specificity of the normal precipitin reaction in plants it is well to consider the variability of the reaction among individuals of a given species. Thus far none of the published accounts have been concerned with the reactions of more than one or a few individuals of a given species. Kostoff does not record the number of repetitions of each normal test. Silberschmidt's data on the subject are so limited as not to afford significant comparative data. Chester's experiments with the woody plants apart from the *Oleaceae* were based on one or very few extracts for each species, and his results with the *Oleaceae* were the only ones published in which a generous assortment of individuals of each species was used. In the latter case, however, although his results show the uniformity of the various individuals and varieties of a species, the fact that the basic reaction here is negative weakens the force of the argument regarding the constancy of the precipitin reactions in a species.

In order to test this matter an experiment was performed by the writers utilizing numerous individuals of a few selected species but under different experimental conditions. Three questions were investigated, namely: What is the effect of age of plant on the precipitin reactions? What is the effect of the addition of calcium and oxalate to the soil upon the precipitin reactions? What variability is there between different horticultural varieties and individuals of a given species? For this experiment cultivated Tomato (*Lycopersicum esculentum*...4 varieties), Pepper (*Capsicum frutescens*...2 varieties), and

Potato (*Solanum tuberosum*...2 varieties) were employed. In the testing of varietal differences all 8 varieties were used, likewise all the varieties were used in testing the effect of addition of salts to the soil, while for the tests on age variations the tomato variety *Bonny Best* was employed. Five or six individuals of each variety were used in each part of the experiment. The experiment on age differences consisted in weekly testing of 5 plants of the tomato variety from the time they were 6-8″ in height to maturity. That on the effect of fertilization consisted in testing the 48 plants used after having treated one third of them with lime, one third with daily applications of 1:1000 potassium oxalate, and leaving one third untreated as controls. The treatments lasted for one month. The tests which were then performed in every case comprised tests with a N/100 potassium oxalate solution, to determine the calcium oxalate reaction, with *Ribes* to determine the effect on the AB reaction, and with *Nicotiana Rusbyi* to observe variations in the MN reaction. The same tubes of *Ribes* and *Nicotiana* were used in all the tests. Unfortunately the *N. Rusbyi* plant selected at the beginning of the experiment, and therefore of necessity employed throughout the whole of the experiment, was negative for the MN reaction. However, the consistency of its negative reactions, under the various experimental conditions is of significance, even though this significance is not as great as though it had been positive. The results of this experiment are gathered in Table XXXVI.

Let us consider first the question of variation among the horticultural varieties of a given species. A comparison of the reactions, taken as a whole, of the four tomato varieties shows them to be entirely uniform with reference to each of the reactions. The average reactivity of the four tomato varieties to oxalate was 1.4, 1.4, 1.2, and 1.8 respectively; that of the four tomato varieties to *Ribes* was 2.0, 1.4, 2.0, and 2.2 respectively; that of the four varieties toward *Nicotiana Rusbyi* was negative in every case. The same reasoning may be applied to a comparison of the two potato varieties with each other and of the two pepper varieties with each other. In addition to these data, the findings of Chester on the reactivity of 18 horticultural varieties of *Syringa vulgaris* may be cited, in which it was shown that when tested under comparable conditions there was no significant variability of reaction among the varieties employed. We are thus free to conclude that all the evidence thus far obtained indicates that the reactions of any selected horticultural variety of a species are approximately equivalent to those of any other selected variety of the same species.

Passing next to the question of variation among individuals of a given

TABLE XXXVI.

A. VARIABILITY OF THE CALCIUM OXALATE, AB AND MN REACTIONS SHOWING EFFECTS OF INDIVIDUAL AND VARIETAL VARIABILITY AND OF SOIL TREATMENTS.

Tomato variety:	Plant #	Treatment:	Precipitin reactions Before treatment: Ox	AB	MN	After treatment: Ox	AB	MN
Pritchard	1	Lime	1	2	0	2	2	0
"	2	Lime	1	2	0	1	2	0
"	3	Oxal.	2	2	0	1	2	0
"	4	Oxal.	2	2	0	2	2	0
"	5	None	1	2	0	1	2	0
Break O' day	1	Lime	1	2	0	2	2	0
	2	Lime	1	1	0	1	2	0
"	3	Oxal.	1	1	0	1	2	0
"	4	Oxal.	2	1	0	1	2	0
"	5	None	2	2	0	1	2	0
Norton	1	Lime	1	2	0	1	2	0
"	2	Lime	1	2	0	2	2	0
"	3	Oxal.	2	2	0	2	2	0
"	4	Oxal.	1	2	0	2	2	0
"	5	None	1	2	0	2	2	0
Bonny Best	1	Lime	1	3	0	1	2	0
"	2	Lime	2	2	0	1	2	0
"	3	Oxal.	3	2	0	1	2	0
"	4	Oxal.	1	2	0	1	2	0
"	5	None	2	2	0	1	2	0

Potato variety:	Plant #	Treatment:	Pptin. reaction after treatment Ca	Ox	AB	MN
Green Mt.	1	Lime	0	1	3	0
"	2	Lime	0	t	3	0
"	3	Oxal.	t	0	3	0
"	4	Oxal.	1	0	2	0
"	5	None	0	0	2	0
"	6	None	0	t	3	0
Var.?	1	Lime	0	t	2	0
"	2	Lime	t	0	2	0
"	3	Oxal.	1	0	2	0
"	4	Oxal.	1	0	2	0
"	5	None	0	0	2	0
Pepper var.						
Red Spot of Oshkosh	1	Lime	1	0	2	0
	2	Lime	1	0	2	0
"	3	Oxal.	1	0	2	0
"	4	Oxal.	1	0	2	0
"	5	None	2	0	1	0
"	6	None	1	0	2	0
Chinese Giant	1	Lime	2	0	2	0
	2	Lime	1	0	2	0
"	3	Oxal.	1	0	2	0
"	4	Oxal.	1	0	2	0
"	5	None	1	0	2	0
"	6	None	1	0	2	0

TABLE XXXVI.

B. VARIABILITY OF THE CALCIUM OXALATE, AB, AND MN REACTIONS IN TOMATO VARIETY "BONNY BEST," ACCORDING TO AGE OF PLANT.

Plant #:	Date of precipitin tests: May 5 Ox	AB	MN	May 11 Ox	AB	MN	May 18 Ox	AB	MN	May 25 Ox	AB	MN	June 1 Ox	AB	MN	June 8 Ox	AB	MN	June 15 Ox	AB	MN
6	2	3	0				2	2	0				2	2	0				1	3	0
7	2	3	0				2	2	0				1	3	0				1	3	0
8	1	3	0				2	3	0				2	3	0				2	2	0
9	2	3	0				2	1	0				1	2	0				1	3	0
10	2	3	0				2	2	0				1	2	0				1	2	0
11	1	2	0				2	2	0				1	2	0				1	2	0
12	1	3	0				2	2	0				2	2	0				2	2	0
13	2	2	0				2	1	0				2	3	0				2	3	0
14	2	2	0				2	2	0				2	3	0				2	3	0
15	2	2	0				2	2	0				1	3	0				1	3	0

variety grown under comparable conditions, we find by consulting Table XXXVI that here too a striking uniformity prevails. The various tests of this table may be divided into 132 pairs, each pair representing a given type of reaction performed on two individuals of the same variety and under comparable experimental conditions. If these 132 pairs of reactions be examined, it will be seen that in 77% of the cases there was no difference in reactivity between the two individual plants of a pair, in 3% of the cases there was only a difference between t and 1, in 19% of the cases there was a difference of one unit between the individuals of a pair, while in only 1% of the pairs was there a difference greater than 1 unit. To these data may be added those of the preceding tables and earlier publications in which it will also be seen that on the whole there is a relatively high degree of agreement between the reactions of individuals of the same species and variety. However, a significant variability has been observed with reference to one of the principles not varied in this table, namely principle M (of *Nicotiana Rusbyi* and *Datura Wrightii*). In our tests as compared with those of Kostoff and Silberschmidt there is evident a certain variability in the occurrence and strength of this reactive principle. M is the least stable of all the reactive groups thus far studied, in conformity with its highly complex nature, and is not a specific character in the same sense as the other reactive principles under observation.

Considering next the variability according to age in the tomato variety Bonny Best, one observes from the table that there is no significant trend of change of reaction strength between the age when testing was first possible (6″ ht.) to the period of maturity. Accordingly we may conclude from the facts at hand that precipitin reactivity is not a variable related to the varying age of the plants tested.

The last question to which this experiment affords an answer is that of the effect of environment on changing the reactivity of individuals. The tomato varieties are all characterized by containing a notable excess of calcium. Treatment of plants of these varieties with lime did not appreciably increase this calcium strength nor did treatment with oxalate appreciably decrease it. The same argument applies to the two varieties of Pepper studied, although in this case the two varieties were characterized by strong excess of oxalate. With regard to the Potatoes, however, a different situation obtains. The potato varieties studied possessed either no excess of calcium and oxalate or only a slight excess of one or the other. They lie on the borderline with respect to the calcium oxalate reaction and accordingly one might expect that here it would be most likely possible to demonstrate an

experimental variation in these principles. Such is the case. With the Potatoes the drastic liming and treatment with oxalate did result in a slight but significant increase of the calcium on the one hand and of the oxalate on the other. We may thus say that the evidence presented justifies the statement that when a species is characterized by a strong calcium or oxalate reaction strong alterations of these elements in the soil fails to produce a demonstrable change in the precipitin reaction, while if a species is characterized by a very weak or negative calcium oxalate reactivity, drastic soil alterations may produce a mild modification of the calcium oxalate reaction. However, such a modification does not seriously disrupt the burden of the significance of the reactions thus far reported since, (a) most species show strong excesses of calcium or oxalate, (b) the soil variation was more drastic than would ordinarily obtain in experimental work on the precipitin reaction, and (c) the differences in reactivity between the controls and the treated plants were not great.

In the light of this experiment we are now able to return to the question of the specificity of the "normal precipitin reaction" and its significance in systematics. We may say that on the whole the experimental data thus far available demonstrate that precipitin reactivity with respect to the various reactive principles studied is a relatively constant species character, that the distribution of the precipitin reactive principles in the various plants tested shows a general agreement with the systematic position of the plants, and that this approximate correlation between presence of reactive principles and taxonomic position is of necessity reflected in a comparable correlation between precipitin reaction and systematics.

As to the practical advantage which may be taken of this correlation, we are now in a better position to judge than before the present analysis was made. It is evident that the precipitin reactions in plants are very different from the precise and specific reactions of animal serology. Hence one cannot consider the two as comparable. One is a protein reaction as far as we know now, the others are far simpler with regard to the nature of the reactive substances but more complex with regard to the number of types of reaction present. The normal precipitin reactions in plants to no extent offer a substitute for the work in the serology of plant proteins. On the other hand, if the taxonomist is interested in studying not only the morphology of his material but also its chemical composition, and recent taxonomic studies have shown the value of such undertakings, then the precipitin technique offers an additional technique of biochemical investigation. It reveals chemical dif-

ferences of varying degrees of significance, although the nature of these differences is not always clear without a chemical analysis, and these differences may have much to offer the investigator of the taxonomy of homogeneous or puzzling plant groups.

E. Immunological Significance of the Precipitin Reactions in Plants

The differences between the precipitin reactions of plants and the reactions of animal serology are so manifest from the preceding experiments that only a brief statement regarding their immunological significance will be introduced at this point.

Of the four reactions which account for all of the interreactions of the 45 species of plants in the present study, no one, so far as we know, is caused by the interaction of protein in one extract with protein in another. The calcium oxalate reaction is a problem in inorganic biochemistry. The AB reaction is due to two interacting substances which, because they are alcohol-soluble, alcohol resistant, and heat resistant, are assuredly non-protein, although they are plainly organic and of large molecular weight. The principle M may be protein in nature but its counter-principle N is relatively simple, apparently crystalloid, and assuredly non-protein. Of the nature of the relatively insignificant XY reaction we know nothing. In the three reactions which we have studied there is no homology with the specific protein reactions of animal serology, in which customarily specific protein reacts with specific protein to produce a precipitation of protein.

It is not inconceivable that other substances than proteins might take part in immunological reactions. Much and Frankel (18) have shown that immunological reactions in animals may be non-specific, Besredka (1) cites instances where they may be localized, and Heidelberger and Avery (10) have shown that immune sera react specifically with a protein-free carbohydrate elaborated by pneumococci. In plants the carbohydrates are much more highly developed than in animals, and it is not impossible that in immunological reactions in plants the carbohydrates would have a much greater rôle than in animals. However, up to the present, the absence of extensive data on this subject requires the utmost caution in formulating theories regarding plant immunological reactions, and the burden of the evidence regarding the nature of the precipitations which have been observed between plant extracts is strongly against any immunological interpretation homologous with that in animal serology. Most assuredly *the work which has been done up to the present on the precipitin reaction in plants does not*

prove that plants can elaborate antibodies as a result of grafting. On the other hand, the *possibility* that plants may be able under certain conditions to elaborate antibodies of the zoöimmunitary type is obviously not eliminated nor is it likely to be with ease because of the relatively small stress which can be laid on negative results in this connection.

F. The Direction of Further Studies in this Field

It is the conviction of the writers that the possibilities in the field of the precipitin reaction in plants are by no means exhausted. Although there is no sound evidence at present demonstrating that, as a result of grafting, plants may acquire or increase their precipitin potency in a zoöimmunitary sense, this thesis is contradicted neither by theory nor by experiment. The absence of normal precipitin reactions in plants does not preclude the possibility of demonstrating acquired reactions, in fact it favors this possibility by eliminating from consideration the artefact non-protein reactions of the types investigated above. It would thus be highly desirable to investigate more graft combinations in this connection, but proceeding in the light of the present study. The number of experiments of this sort which could be performed must necessarily be limited because of the necessity, in passing, of investigating the nature of the reactions found. The biochemical studies reported above will serve as an outline of the procedures found desirable in investigating the nature of such reactions. Certainly no graft reaction should be described as immunological in the zoöimmunitary sense in which the reactive substances are not investigated at least with regard to their solubilities, their behavior in the presence of heat and of strong alcohol, and their relation to dialyzing membranes. In view of the differences manifested by the precipitations in animals and plants an unlimited application of serological terminology to the plant phenomena is to be discouraged. It engenders in the minds of workers in related fields a belief that the reactions in the two fields are homologous. Carbone's term "pseudo-antibodies," although not wholly expressive of the situation, is far preferable to an uncritical use of the terms precipitin, lysin, antigenic extract, etc., and for this reason the writers, here and elsewhere, have attempted to minimize this misconception, where possible, by avoidance or quotation of such terms.

VIII. SUMMARY

The present paper reports an analysis of the nature of the "normal precipitin reaction" in plants. The conclusions reached are based upon more than 4000 precipitin tests, heretofore unpublished, using 45 species

of herbaceous and woody plants as material. Proceeding from an analysis of the reaction between *Prunus*, *Platanus*, *Ribes*, *Robinia*, and *Hydrangea*, the scope of the analysis was extended to include the various interreactions of other woody plants and of 35 of the *Solanaceae*. The results obtained may be summarized in the following points:

1. The interreactive substances of *Prunus*, *Platanus*, *Ribes*, *Robinia*, and *Hydrangea* are characterized by the following properties:
   a. They are coctostable (3 hrs. autoclaving at 0-2 lbs. pressure).
   b. They are relatively insensitive to variations of pH within wide limits. Strong alkalinization or acidification of the extracts does not remove the reactive substances, although the reaction does not occur at very low or very high pH values.
   c. The reactive substance of *Prunus* is not affected by variation of salt concentration within wide limits, that of *Platanus* and *Robinia* steadily decreases in strength as the salt (phosphate) content increases.
   d. On dilution of the extracts there is no evidence of a zone phenomenon such as occurs in animal serology.
   e. The interreactive substances of these plants pass freely through dialyzing membranes highly impermeable to protein.
   f. The interreactive substances in these plants are equally soluble in water and in physiological saline; they are insoluble in strong alcohol, ether, chloroform, carbon tetrachloride, benzol, and 95% alcohol + 1% tartaric acid; they are not denatured by any of these solvents; and the reactions take place with equal facility in the presence of NaCl (.85%), MgO, and N/20 NaOH.
   g. The reactive ingredient in *Prunus* is precipitated by neutral lead acetate but is recoverable in such precipitates, that of the other species is not so precipitable and is reclaimable in the practically carbohydrate-free fraction resulting from neutral and alkaline lead acetate precipitation.
   h. Double precipitation shows that only a single type of reaction is involved between these species.
2. That this reaction is due to the interaction of free oxalate in *Prunus* with free *calcium* in the other extracts has been demonstrated:
   a. By qualitative analysis (the results of which are summarized on page 159).
   b. By quantitative analysis which showed calcium oxalate to be present in the washed precipitates to the extent of 85-90%.
   c. By complete elimination of the reactions by precipitating *Prunus*

with calcium salts or the other extracts with oxalates respectively.

3. This calcium oxalate "precipitin reaction" has been found to occur extensively throughout the woody plants and *Solanaceae* studied. About half of all the reactions obtained in this study were positive, and of these positive reactions 57% were due to calcium oxalate. 70% of our positive tests of the plants used by Kostoff in this work were found to be due to calcium oxalate.
4. By now eliminating the calcium oxalate reaction from consideration it was possible to determine whether other reactions were present among these species. Three such other reactions have been distinguished. One, the AB reaction, is characterized by the reactions of the *Rosiflorae* employed (A) with the *Solanaceae* (B). It accounts for 24% of all the positive tests observed in our material. A second reaction, MN, particularly results from the interaction of *Nicotiana Rusbyi* and *Datura Wrightii* (M) with other *Solanaceae* (N). It is responsible for 17% of our precipitates. The third reaction, XY, occurs between the *Oleaceae* (Y) studied and certain of the *Solanaceae* (X). It is relatively insignificant, being responsible for only 2% of the total reactions observed. The identity and distinctness of these three reactions have been established by various types of experiment.
5. Principles A and B are water-soluble, alcohol-soluble, coctostable, insoluble in lipoid solvents, not coagulable by alcohol, and slowly and imperfectly dialyzable. The precipitate formed from their interaction chars upon ignition. Hence they are plainly non-lipoid and non-protein (because of their alcohol solubility and positive, though imperfect dialysis) but organic and of relatively high molecular weight. Principles M and N are soluble in water and weak salt solutions, but are insoluble in alcohol, are non-coagulable by alcohol, insoluble in lipoid solvents, and in contrast to A and B are thermolabile. The precipitate formed by their interaction chars on ignition. M is retained by dialyzing membranes with high efficiency, but N passes freely through such membranes. Hence they are both organic and non-lipoid. N is in all probability an organic substance of relatively simple structure and it is highly unlikely that it is protein. M, on the other hand, gives strong indications of being protein or at least a complex organic substance of high molecular weight.
6. The published immunological interpretations of precipitin reactions in plants are rendered untenable because of the lack of homology

between the animal and plant reactions, and because of the widespread occurrence in the plants heretofore used of simple, non-protein reactions. The works of Kostoff, Silberschmidt, and East are analyzed in this connection.

7. The findings of Chester with regard to the reactions of diseased Lilacs and to the specificity displayed by the normal reactions are interpreted and substantiated by these findings.

8. The experimental work has shown that the age of the plants does not have a significant effect upon the strength of the calcium oxalate reaction. Treatment of tomato and pepper varieties with lime, and with potassium oxalate did not appreciably alter the strength of the calcium oxalate reaction. The same treatment applied to potato varieties produced a demonstrable change in this reaction. In the later case the slight effect produced by experimental variation of the environment cannot be considered as seriously affecting the significance of the reactions reported.

9. The possibility, in the future, of demonstrating immunological reactions from grafting is not eliminated by this study. There is proof that the reactions thus far reported were doubtless non-immunological in the zoöimmunitary sense, but on the basis of the findings of the present study the desirable directions of future work in this field are pointed out.

IX. ACKNOWLEDGEMENT

The work reported in the present paper has been very appreciably furthered by the assistance and suggestions of a number of workers in related fields. The experiments in chemical analysis of the extracts were materially aided by the coöperation of Drs. Ronald Ferry and Benjamin White. For numerous suggestions and criticisms throughout the study the writers are indebted to Professors J. H. Faull and E. M. East. To them the writers wish, at the present time, to express their thanks.

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NEW SPECIES, VARIETIES AND COMBINATIONS FROM THE HERBARIUM AND THE COLLECTIONS OF THE ARNOLD ARBORETUM[1]

ALFRED REHDER

With nine text figures

Celtis Rockii, sp. nov.

Arbor 6-metralis, ramulis hornotinis dense flave crispulo-villosis angulatis vel subangulatis lenticellatis, annotinis tarde glabrescentibus; gemmae ovoideae, acutae, perulis adpresse flavo-pilosis. Folia papyracea, elliptico- vel rhombico-ovata, 4-8 cm. longa et 2.3-4.5 cm. lata, breviter acuminata, basi plus minusve oblique late cuneata vel fere rotundata, a medio vel infra medium ad apicem dentato-serrata, supra accumbenti-pilosa et scabrida, subtus tota facie satis dense molliter crispulo-villosa, ad nervos pilis longioribus patentibus flavidis instructa, triplinervia, nervis utrinsecus basalibus inclusis 2-3; petioli flave crispulo-villosi, 3-5 mm. longi. Flores non visi. Racemi fructiferi axillares in parte inferiore ramulorum plerique triflori, pedicello terminali incluso 1-1.5 cm. longi, dense flave crispulo-villosi, pedunculo 2-5, pedicellis 3-8 mm. longis; drupa subglobosa, parva, plus minusve villosula, vel maturitate glabra vel fere glabra, lutea (ex collectore) in sicco atro-fusca; putamen subglobosum, circ. 4 mm. diam., manifeste punctulato-foveolatum et leviter costatum.

CHINA. Yunnan: region of Tungshan, Yangtze drainage basin, east of Likiang, *J. F. Rock*, no. 10522 (type), in 1923 (tree 20 ft.; fruits yellow).

This new species seems closely related to *C. Salvatiana* Schneid., and also to *C. cinnamomea* Lindl., but from both it is easily distinguished by the rather dense pubescence of the leaves, the densely pubescent branchlets and inflorescence and the pubescent fruit; from *C. cinnamomea* it differs further in the serrate broader leaves, the few-flowered short-peduncled inflorescence and smaller fruit.

[1]Continued from vol. XIII. 341.

Clematis chinensis Osbeck, Dagb. Ostind. Resa, 205, 242 (1757); Reise Ostind. China, 267, 315 (1765); Voy. China East Ind. I. 329; II. 356 (1771).—Retzius, Observ. II. 18, t. 2 (1781).—De Candolle, Syst. I. 137 (1818); Prodr. I. 3 (1824).—Forbes in Jour. Bot. XXII. 262 (1884).—Hemsley in Jour. Linn. Soc. XXIII. 3 (1886).—Pritzel in Bot. Jahrb. XXIX. 332 (1900).—Finet & Gagnepain in Bull. Soc. Bot. France, L. 535 (1903); Contrib. Fl. As. Or. I. 20 (1905), excl. synon. *C. terniflora* et *C. Benthamiana*.—Rehder & Wilson in Sargent, Pl. Wilson. I. 329 (1913); in Jour. Arnold Arb. VIII. 106 (1927).—Léveillé, Fl. Kouy-Tchéou, 332 (1915).—Merrill in Jour. Am. Bot. III. 579 (1916).—Rehder in Jour. Arnold Arb. X. 187 (1929).

Clematis sinensis Loureiro, Fl. Cochinch. 345 (1790).

Clematis minor Loureiro, l. c. (1790).—De Candolle, Syst. I. 136 (1818); Prodr. I. 3 (1824).—Forbes in Jour. Bot. XXII. 263 (1884).

Clematis recta § *chinensis* Kuntze in Verh. Bot. Ver. Brandenb. XXVI. 114 (Monog. Clem.) (1885).

Clematis funebris Léveillé & Vaniot in Bull. Acad. Intern. Géog. Bot. XI. 168 (1902).

Clematis oligocarpa Léveillé & Vaniot, l. c. XVII. no. 210-11, p. ii (1907).—Léveillé, Fl. Kouy-Tchéou, 333 (1915).

Clematis Cavaleriei Léveillé & Porter in Fedde Rep. Spec. Nov. IX. 20 (1910).—Léveillé, Fl. Kouy-Tchéou, 332 (1915).

The fact that the generally accepted name *Clematis chinensis* Retzius would be invalidated by the older homonym *C. chinensis* Osbeck, if the two names referred to different species, has led me to investigate this question and I find that the two names are synonymous, as they have been already treated by a few authors. This will save the specific epithet *chinensis* for the species generally known under this name, and makes necessary the change of the author citation only, so that it will be *C. chinensis* Osbeck (1757) instead of Retzius (1781).

Owing to the fact that Osbeck's name was published with a rather incomplete description hidden away in the text of a work not primarily taxonomic, it has been neglected, while Retzius' name published with an adequate description accompanied by a plate has been universally accepted. Also the misleading citation in Index kewensis of *C. chinensis* Osbeck as a synonym of *C. recta* L. may have caused the disregard of the name by later authors. Moreover E. D. Merrill (l. c.) has tried to identify *C. chinensis* Retz. with *C. Meyeniana*, but the description given by Osbeck (l. c. p. 205) "Plurima habet communia Clematide Vitalba, at folia lanceolata, angustissima, & flores minores" agrees much better with *C. chinensis* Retz. which has pinnate leaves like *C. Vitalba* with narrower leaflets and decidedly smaller flowers, while *C. Meyeni-*

ana has ternate leaves with large subcoriaceous leaflets, quite different from the pinnate leaves of *C. Vitalba*, and flowers scarcely smaller than those of the latter species. Additional data given by Osbeck (l. c. p. 242) "Pistilla 3 ad 6, stylis plumosis in orbem positis reflexis. Stam. O obseruavi. Frutex scandens, ramosissimus" may apply as well to *C. chinensis* as to *C. Meyeniana*. In his more recent Commentary on Loureiro's Flora cochinchinensis (msc.), however, Merrill identifies *Clematis minor* Lour. with *C. chinensis* Osbeck and states that according to his opinion *C. chinensis* Retz. and *C. chinensis* Osbeck are identical; he also considers *C. Benthamiana* Hemsl. a synonym, following Finet & Gagnepain, which I keep separate (see Rehder & Wilson, l. c.).

The identity of *C. chinensis* of Osbeck and of Retzius is proven conclusively by specimens before me, for the loan of which I am indebted to those in charge of the herbarium of the State Museum in Stockholm. One of these specimens is marked on the back of the sheet "China: Osbeck," a second "Herb. Swartzii—Osb." and a third "ex Ind. Orient.," the last note may have reference to the fact that it was collected during Osbeck's East Indian voyages, but as the species does not occur in East India, the specimen probably came from China. All these specimens represent very early collections and were probably all collected by Osbeck; they are all named *Clematis chinensis* Retz., the determinations being apparently of a later date, for two of them bear the citation "DC." The specimens vary somewhat in the shape and size of the leaves and only one has leaflets as narrow and small as in Retzius' plate of *C. chinensis*.

Clematis chinensis is widely distributed in southeastern and central China extending west to Szechuan and Kweichou. In this herbarium there are specimens from the following provinces: Kwangtung, Fukien, Hunan, Anhwei, Chekiang, Kiangsi, Hupeh, Szechuan and Kweichou; there is also a specimen from Annam, and a photograph of the type of *C. minor* Lour. from Cochinchina.

Clematis grata Wall. var. **likiangensis,** var. nov.

A typo recedit achaeniis glabris.—Ramuli, petioli et inflorescentiae laxe villosula. Foliola ovata, trilobata lobis grosse paucidentatis, supra glabrescentia, subtus in costa venisque densius, in venulis sparsius et in facie sparsissime flavido-pilosis; flores ut in typo carpellis glabris exceptis.

China. Yunnan: Yangtze watershed, Prefectural district of Likiang, eastern slopes of Likiang Snow Range, *J. F. Rock*, nos. 3668 (type) and 3918.

Clematis grata apparently varies like the related *C. Gouriana* Roxb.,

and *C. brevicaudata* DC. with pubescent and glabrous akenes (*C. Gouriana* var. *Finetii* Rehd. & Wils. and *C. brevicaudata* var. *lissocarpa* and var. *subsericea* Rehd. & Wils.), which shows that the pubescence of the akenes is a character of secondary importance and cannot be used to define subdivisions of the genus.

Deutzia Esquirolii (Lévl.), comb. nov.

Styrax Esquirolii Léveillé in Fedde Rep. Spec. Nov. IX. 446 (1911).

Deutzia lancifolia Rehder in Sargent, Pl. Wilson. I. 147 (1912); Jour. Arnold Arb. XII. 276 (1931).—Léveillé, Fl. Kouy-Tchéou, 387 (1915).

Deutzia Chaffanjoni Léveillé, l. c. (1915), pro synon. *D. lancifoliae* Rehd.

Deutzia Esquirolii (Lévl.) Léveillé, l. c. (1915), pro synon. *D. lancifoliae* Rehd.

When dealing with *D. lancifolia* in my Notes on the ligneous plants described by Léveillé (Jour. Arnold Arb. XII. 276) I overlooked that unfortunately Léveillé's *Styrax Esquirolii* is one year older than my *Deutzia lancifolia* and that the new combination resulting from the transfer of the specific epithet of his name should be the valid name for the species. Léveillé himself had already published this combination, but only as a synonym of *D. lancifolia*.

Hydrangea umbellata Rehder in Sargent, Pl. Wilson. I. 25 (1911).

Hydrangea Schindleri Engler in Engler & Prantl, Nat. Pflanzenfam. ed. 2, XVIII-A, 203 (1930), pro parte.—**Synon. nov.**

Hydrangea Schindleri was only briefly mentioned in Engler's account of the species of *Hydrangea* (l. c.) without enumeration of specimens. Engler compared it with *H. chinensis* Maxim. and *H. umbellata* Rehd. In the Berlin Herbarium there are four specimens labeled *H. Schindleri* all collected by A. K. Schindler in August-September 1908 at Lu-shan, Kuling mountains, Kiangsi, the type locality of *H. umbellata*. Two of them, nos. 325 and 327, I cannot distinguish from *H. umbellata* while the other two numbers belong to the following species:

Hydrangea paniculata Siebold in Nov. Act. Acad. Leop.-Carol. XIV. pt. II. 690 (Syn. Hydr.) (1829).

Hydrangea Schindleri Engler in Engler & Prantl, Nat. Pflanzenfam. ed. 2, XVIII-A, 203 (1930), pro parte.— **Synon. nov.**

Of the four numbers collected by Schindler at Lu-shan, Kiangsi, and named by A. Engler *H. Schindleri* two belong to the preceding species, while the other two, nos. 322a and 324, are identical with *H. paniculata* Sieb. No. 324 bears the following note in A. Engler's handwriting, "Hydrangea Schindleri Engl. n. sp., affinis Hydr. chinensi Maxim., differt foliis ab infima triente sursum angustatis, haud e medio utrinque angustatis, distinctius serratis, florum sterilium sepalis ovatis angusti-

oribus." This seems to show that this specimen should be considered the type of *H. Schindleri* Engl., since the preceding characterization is apparently based on Schindler's no. 324 rather than on any of the other numbers and is the same as given in German in the Pflanzenfamilien.

In 1911 in my Synopsis of the Chinese species of Hydrangea (in Sargent, Pl. Wilson, I. 25, 1911) I stated that Wilson's no. 1601, collected at Kuling, July 27, 1907, was to my knowledge the first specimen of *H. paniculata* collected in China. Since then, however, many additional specimens have come to this herbarium and the species is now known from the following Chinese provinces: Kiangsu: Yii-du-hsien, *H. H. Hu*, no. 1179. Anhwei: Chu-hwa-shan, *R. C. Ching*, no. 2808; Wu-yen, *N. K. Ip*, no. 7675. Chekiang: Tsing-Tien, Taishun-hsien and Chang-shan-hsien, *Y. L. Keng*, nos. 172, 310 and 841; Pang-yung, *R. C. Ching*, no. 2099; East Tien-mu, *H. H. Hu*, no. 1609. Kiangsi: Kuling, *E. H. Wilson*, no. 1601; Lu-shan (Kuling), *A. K. Schindler*, nos. 322a, 324; Lu-shan, *A. N. Steward*, no. 2613; Ningdu, *Wang-Te-Hui* in Handel-Mazzetti, Pl. Sin., no. 442. Fukien: Yenping, *H. H. Chung*, nos. 2844, 3301, 3556 and 3659. Kwangtung: Lokchong, *Y. Tsiang*, no. 1219; between Bei-shen and Nan-shung, *W. Y. Chun*, no. 5683; road to Chang-kiang, *W. Y. Chung*, no. 5794; Siudsao, *R. Mell*, no. 1773. Hunan: Wukang, *Handel-Mazzetti*, no. 12527. Kweichou: Lou-tsong-koan, *E. Bodinier*, no. 1661; Kwei-yang, *Handel-Mazzetti*, no. 10478; Kweiting, *Y. Tsiang*, no. 5627. Yunnan: Yunnanfu, *O. Schoch*, no. 423. The specimen collected in 1897 by E. Bodinier in Kweichou was described by Léveillé as *H. Kamienskii* (cf. Jour. Arnold Arb. XII. 277).

Spiraea yunnanensis Franchet, Pl. Delavay. 200 (1890).—Schneider, Ill. Handl. Laubholzk. I. 463 (1905).

Spiraea sinobrahuica W. W. Smith in Not. Bot. Gard. Edinb. x. 67 (1917); XIV. 233, 260 (1924); XVII. 388 (1930).—**Synon. nov.**

Spiraea sinobrahuica var. *aridicola* W. W. Smith in Not. Bot. Gard. Edinb. x. 68 (1917); XVII. 197, 363 (1930).—**Synon. nov.**

CHINA. Szechuan: in valle fl. Ming, inter stationes Sim-puguanj et Pei-schuy-tchan, *G. N. Potanin*, Aug. 25, 1873 (frutex usque metralis); inter Tatsien-lu et Batang, ad stationem Natschuka sive Nachtschuka, *V. Kashkarov*, May 19, 1893 (frutex plus quam metralis); between Batang and Tschien-lu, *John R. Muir*, in 1911; Muli kingdom, Shou-chu valley, alt. 2435-2900 m., *J. F. Rock*, no. 16279, June 1928. (shrub 1-1.5 m.) Yunnan: terrains calcaires, pierreux au dessus de Mo-so-yn, Lankong, alt. 2200 m. *J. Delavay*, no. 1082, (holotype of *S. yunnanensis*, photo. and fragments in A. A.), May 1,

1884 (arbrisseau d'un mètre; fleurs blanches); eastern flank of the Lichiang range, Lat. 27° 10′ N., alt. 9000-10500 ft., amongst the scrub in side valleys, *G. Forrest, no.* 5580 (syntype of *S. sinobrahuica;* ex W. W. Smith, l. c.); descent of the Yangtze from the eastern boundary of the Lichiang valley, lat. 27° 15′ N., alt. 9000-10000 ft., *G. Forrest,* no. 10117 (syntype of *S. sinobrahuica*), June 1913 (shrub 4-5 ft.; flowers creamy white); descent of the Yangtze valley from the eastern range of the Lichiang valley, Lat. 27° 30′ N., alt. 9000-10000 ft., *G. Forrest,* no. 10084 (syntype of *S. sinobrahuica* var. *aridicola*), June 1913 (shrub 4-6 ft.; flowers creamy white); mountains of Chungtien plateau, lat. 27° 30′ N., alt. 11000 ft., *G. Forrest,* no. 12634 (syntype of *S. sinobrachuica* var. *aridicola;* photo. in A. A.); open stony slopes and on ledges of dry cliffs on the western flank of the Lichiang range, Lat. 27° 40′ N., Long. 100° 18′ E., alt. 10-11000 ft., *G. Forrest,* no. 21171, May 1922 (shrub 3-5 ft.; flowers creamy-white); Yangtze valley, northwest of Likiang, Lat. 27° 20-30′ N.; alt. 2000-2100 m., *Handel-Mazzetti,* no. 8792, June 2, 1916; Yangtze watershed, Prefectural district of Likiang, eastern slopes of Likiang snow range, *J. F. Rock,* no. 3639, May-Oct. 1922; western slope of Likiang snow range, Yangtze watershed, *J. F. Rock,* no. 8557, April 1923 (shrub forming globose bushes); Lotueshan, mountains of Labako, west of Yangtze bend at Shiku, *J. F. Rock,* no. 8471, April 1923 (shrub 3-4 ft.; flowers white); dry rocky slopes and on cliffs on the Chien-chuan-Mekong divide, Lat. 26° 36′ N., Long. 99° 40′ E., alt. 9-10000 ft., *G. Forrest,* no. 21465, July 1922 (shrub of 2-3 ft.; flowers white); open dry rocky slopes and ledges of cliffs on the Mekong-Salween divide, Lat. 28° 12′ N.; alt. 7-9000 ft., *G. Forrest,* no. 16410, May 1918 (shrub 1-3 ft., flowers creamy white). Southeastern Tibet: Tsarong, open stony situations on the ledges of cliffs on the Salween-Kiu-chiang divide, Lat. 28° 40′ N., alt. 7000 ft., *G. Forrest,* no. 18881, July 1919; on ledges and in crevices of cliffs and dry bouldery slopes on the Salween-Kiu-chiang divide, Lat. 28° 40′ N., Long. 98° 15′ E., alt. 8000 ft., *G. Forrest,* no. 19147, Sept. 1919 (shrub 1-2 ft., widely branched).

I have been unable to find any characters to separate *Spiraea sinobrahuica* W. W. Sm. from *S. yunnanensis* Franch. The chief difference given by the author of the former name, the glabrous upper surface of the leaves of *S. yunnanensis,* does not hold, since the leaves of the type specimen of *S. yunnanensis* show the same pubescence of short accumbent hairs on their upper surface as the types of *S. sinobrahuica,* though Franchet describes them as glabrous, a statement which induced the author of *S. sinobrahuica* to consider Forrest's specimens distinct. It also does not seem possible to separate var. *aridicola* from the type.

The species which is closely related to *S. brahuica* Boiss. is very variable in the size, in the serration and to some extent in the shape of the leaves, in the size of inflorescence and in habit; the two extremes merge imperceptibly into each other and are apparently only individual differences caused by difference of exposure and soil. The only form which seems worthy of a distinct name on account of its striking habit is the following.

Spiraea yunnanensis f. **tortuosa** (Rehd.), forma nova.

Spiraea tortuosa Rehder in Sargent, Pl. Wilson. 1. 445 (1913).

CHINA. Szechuan: Mao-chou, arid regions of the Min valley, alt., 13-2000 m., *E. H. Wilson*, no. 2764 (holotype of *S. tortuosa*), May 25, 1908 (shrub 3-4 ft.; flowers white).

This differs strikingly from the type in its distinctly zigzag branchlets, the perfectly straight internodes forming sharp angles of about 130-150° with each other; the leaves are suborbicular to broadly oval, more or less 3-lobed and scarcely exceed 12 mm. in length; the inflorescence is 5-12-flowered. The only specimens enumerated under the type which show any approach to zigzag branchlets, are Potanin's specimen from the same region and John R. Muir's specimens from western Szechuan. The specimens described as *S. sinobrahuica* var. *aridicola* may be considered as being nearest to f. *tortuosa*.

Spiraea siccanea (W. W. Sm.), spec. nov.

Spiraea yunnanensis Fr. var. *siccanea* W. W. Sm. in Not. Bot. Gard. Edinb. x. 69 (1917).

Frutex 1-1.5 m. altus ramis gracilibus teretibus, hornotinis adpresse villosulis, annotinis glabris purpureo-fuscis partim decorticantibus; gemmae ovoideae, pluriperulatae perulis ovatis glabris ciliolatis. Folia ramulorum floriferorum (turionum non vidi) obovata vel ovalia, rarius oblongo-ovalia, 0.8-2 cm. longa et 6-10 mm. lata, apice rotundata vel obtusa, mucronulata, basi late cuneata vel fere rotundata, supra medium inaequaliter dentata vel crenato-dentata dentibus mucronulatis, interdum indistincte trilobata, supra laete viridia et glabra, subtus glauca ad costam nervosque tantum laxe pilosa, basi 3- vel 5-nervia, ceterum utrinque nervis 1-2 instructa; petioli 1-2 mm. longi, villosuli. Flores albi, subumbellati, circiter 12-20 ramulos paucifoliatos pedunculo 5-10 mm. longo incluso 1-2 cm. longos terminantes; pedicelli 5-10 mm. longi ut pedunculus villosuli; calyx turbinatus, circ. 1 mm. longus, ut lobi triangulares acutiusculi subaequilongi laxe villosus; petala orbiculari-obovata, 3-4 mm. longa; stamina circ. 20, dimidia petala aequantia; discus conspicuus, annularis, 10-lobatus; carpidia villosula, stylo apicali fere 1 mm. longo coronata. Fructus non vidi.

CHINA. Yunnan: Lang-kong-Hoching mountains, Lat. 26° 16′

N., alt. 8000 ft., open dry situations, *G. Forrest*, no. 9912 (syntype of *S. yunnanensis* var. *siccanea*), May 1913 (shrub of 3-5 ft.; flowers white); Lang-kong-Hoching divide, Lat. 26° 10′ N., alt. 8000 ft., dry stony situations amongst scrub, *G. Forrest*, no. 9972 (syntype of *S. yunnanensis* var. *siccanea*) May 1913 (shrub of 3-5 ft.; flowers creamy white).

This seems to be distinct enough to be specifically separated from *S. yunnanensis* Franch. from which it is readily distinguished by the leaves being quite glabrous above and only loosely pilose on the veins beneath; no forms intermediate in pubescence were found among the numerous specimens seen of *S. yunnanensis*, except perhaps one specimen collected between Batang and Tachienlu by John R. Muir, in which the pubescence though rather slight, is present nevertheless on both sides; at the same time the specimen has very small, 4-8-flowered inflorescences, very small leaves and slightly tortuous branchlets.

Malus Rockii, sp. nov.

Arbor 8-10 m. alta, ramis pendulis; ramuli hornotini villosi, annotini glabrescentes fusci vel fusco-rubri; gemmae ovoideae, perulis atrofuscis ovatis medio villosis ceterum fere glabris. Folia chartacea, elliptica vel ovato-elliptica, ovata ad ovato-oblonga, 6-12 cm. longa et 3.5-7 cm. lata, acuminata, basi rotundata, rarius late cuneata, argute et adpresse inaequaliter serrulata, dentibus mucronato-acuminatis, supra costa sparse villosa excepta glabra et in costa et nervis glandulosa, impresso-reticulata, subtus pallida, in costa, nervis et venulis manifeste elevatis crispo-villosa, interdum sparse in facie villosula, venulis trabecularibus conspicuis; petioli 2-4 cm. longi, villosi. Flores non visi. Fructus 2-4 vel solitarii, pedicellis 2-4 cm. longis villosis suffulti, ovoidei vel subglobosi, basi in petiolum abrupte attenuata, apice juniores tandem plus minusve leviter attenuati 1-1.5 cm. longi, calyce tarde deciduo, juniores apice et basi villosuli lobis calycinis lanceolatis extus intusque villosis partim coronati, maturitate carminei, luciduli, 5-loculares.

CHINA. Yunnan: west of Talifu, Mekong watershed, en route to Young-chang and Tengyueh beyond Lampba, along watercourses, alt. 7000 ft., *J. F. Rock*, no. 6842 (type), Sept.-Oct. 1922 (tree with long drooping branches; fruits carmine, cherry-like); Litiping range, Mekong-Yangtze divide, east of Weihsi, *J. F. Rock*, no. 11552, Oct. 1923 (tree 25 ft.); Yangtze watershed, western slopes of Likiang Snow Range, *J. F. Rock*, no. 5346, May 30-June 6, 1922 (tree 35 ft.).

This new species is apparently nearest to *M. baccata* (L.) Borkh. but the fruits are larger, the calyx is tardily deciduous and the leaves are rather densely pubescent and reticulate beneath. From *M. pumila*

Mill. which it resembles somewhat in its leaves, it is farther removed by the deciduous calyx and the slender-stalked small fruit not impressed at the base and the apex; also the leaves are more strongly reticulate and rounded at base. One might compare *M. Rockii* with the hybrids between *M. baccata* and *M. pumila* or *M. prunifolia* (Willd.) Borkh., but the calyx seems to be always deciduous and the leaves are pubescent and reticulate beneath; moreover, hybrids between these two northern species cannot be expected to occur in Yunnan even as escapes from cultivation, and the three specimens cited above are apparently from spontaneous trees.

Malus hupehensis (Pamp.), comb. nov.

Pirus communis Pavolini in Nuov. Giorn. Bot. Ital. xv. 415 (1908).—Non Linnaeus.

Pirus hupehensis Pampanini in Nuov. Giorn. Bot. Ital. n. ser. xvii. 291 (1910).—Rehder in Sargent, Pl. Wilson. ii. 265, 300 (1915).

Pyrus baccata Hemsley in Jour. Linn. Soc. xxiii. 255 (1886), quoad plantam e Chekiang.—Diels in Bot. Jahrb. xxix. 387 (1900).—Non Linnaeus.

Pyrus spectabilis Hemsley in Jour. Linn. Soc. xxiii. 258 (1886), quoad plantam e Kiangsi et Hupeh.—Diels in Bot. Jahrb. xxix. 387 (1900).—Non Aiton.

Malus baccata var. *himalaica* Schneider, Ill. Handb. Laubholzk. i. 721, fig. 397s (1906), quoad plantam chinens. et fig.—Non Pyrus baccata var. himalaica Maxim.

Malus theifera Rehder in Sargent, Pl. Wilson. ii. 283 (1915); in Jour. Arnold Arb. v. 192 (1924); viii. 121 (1927); Man. Cult. Trees Shrubs, 395 (1927).—Chun, Chin. Econ. Trees, 173, fig. 65 (1922).—Hers in Jour. N. China Branch R. As. Soc. liii. 116 (1922); Liste Ess. Lign. Honan, 29 (1922).—Chung in Mem. Sci. Soc. China, i. 82 (Cat. Trees Shrubs China) (1924).—Hu & Chun, Icon. Pl. Sin. i. 32, t. (1927).—Wilson in Arnold Arb. Bull. ser. 3, i. 20, fig. (1927).

Pyrus theifera (Rehd.) Bailey in Rhodora, xviii. 155 (1916); Stand. Cycl. Hort. v. 2872 (1916).—Kew Handlist Trees Shrubs, ed. 3, p. 133 (1925).

It is rather unfortunate that the name of this species which as *Malus* or *Pyrus theifera* is already well known in horticultural literature as a highly ornamental Crabapple, has to be changed, but when examining last year in the Biondi herbarium at the Botanical Museum in Florence the type of Pampanini's *Pirus hupehensis*, I saw at once that this species is identical with my *Malus theifera*. When describing the latter species I had not seen a specimen of Pampanini's species, which according to the author's remarks was most closely related to *P. pashia* Buch.-Ham. and also to *P. communis* L. Owing to the world war, I was unable to obtain a specimen of the species from Florence and I, therefore,

mentioned (l. c. 265, 300) *P. hupehensis* among the doubtful species of *Pyrus*, but stated that it could not belong to *Pyrus* in the restricted sense, since the author described it as having three connate styles villous below. Later Pampanini apparently revised his opinion regarding the affinity of this species, since on a note dated December 1921 and pinned to the sheet of each type specimen he referred it to *Malus baccata* var. *himalaica* Schneid., making a new binomial combination of that variety under *Pirus* which, however, was never published. This identification, of course, came much closer to the true relationship of his *P. hupehensis*.

Malus hupehensis is widely distributed in mountainous regions of China at elevations of from 1000-2000 m. and extends south into Assam. It is represented in this herbarium by specimens from the following Chinese provinces: Shantung, Kiangsu, Honan, Chekiang, Kiangsi, Fukien, Hunan, Hupeh, Szechuan, Kweichou and Yunnan; also from Assam. The syntypes of *P. hupehensis* Pamp. were collected in northern Hupeh, Sian-men-kou (Silvestri, no. 939) and Ma-pau-scian (Silvestri, nos. 940, 9402); of nos. 939 and 940 there are photographs in this herbarium. The holotype of *M. theifera* also came from Hupeh, near Ichang (Wilson, no. 451), and the paratypes from other localities in Hupeh, from Shensi, Chekiang, Szechuan and Assam.

A form with rosy-pink flowers is the following:

Malus hupehensis f. **rosea** (Rehd.), comb. nov.

Malus theifera f. *rosea* Rehder in Sargent, Pl. Wilson. II. 284 (1915); Man. Cult. Trees Shrubs, 395 (1927).

Pyrus theifera var. *rosea* (Rehd.) Bailey in Rhodora, XVIII. 155 (1916); Stand. Cycl. Hort. v. 2872 (1916).

This form has been found in Hupeh; the type comes from Fang Hsien, (Wilson, no. 2980) and a paratype from Patung Hsien (Wilson, Veitch Exped. seed no. 766, Oct. 1900; specimen from Kew Bot. Gard., Wm. Bean, May 1914).

Prunus Slavinii Palmer, hybr. nov.

Prunus angustifolia var. *varians* Wight & Hedrick × *P. gracilis* Engelm. & Gray.

Frutex, 4-12 dm. altus, raro arborescens et ad 2-2.5 m. altus, dense ramosus, ramulis spinescentibus, novellis brunneo-rubescentibus glabris vel pubescentibus, vetustioribus cinereo-brunneis. Folia lanceolata vel ovato-lanceolata, tenuia sed firma, supra fere glabra, infra glabra vel pubescentia, venulis reticulatis; petioli graciles, 1-1.5 cm. longi glabri vel pubescentes, eglandulosi vel raro glandulosi. Flores 2-6-umbellati, pedicellis 9-12 mm. longis glabris vel pubescentibus. Fructus ovoideus vel subglobosus, 1.5-2.2 cm. longus, 1-2 cm. latus, ruber, pallide punctatus, vel rubro-luteus, succosus, esculentus.

Slender or arborescent shrubs .5 to 1 m. or rarely 2 to 2.5 m. tall, with numerous spreading or ascending somewhat spinescent branches, those of the last year's growth reddish-brown. Bark on old stems and branches dark gray or gray-brown, with pale lenticels. Leaves lanceolate or ovate-lanceolate 3.5-7 cm. long, 1-2.5 cm. wide, rounded or slightly subcordate at base, rounded, acute or short-acuminate at apex, finely serrate with shallow gland-tipped teeth, bluish-green and glabrous or sparsely pubescent above, paler and usually more densely pubescent beneath, sometimes only along the prominently reticulate veins, thin but firm in texture, on slender eglandular or rarely glandular petioles. Flowers appearing in March or early April before the leaves in 2-6-flowered umbels, on slender pubescent or nearly glabrous pedicels; ovary usually somewhat pubescent, rarely glabrous; calyx-teeth lanceolate, usually with entire margins, glabrous or slightly pubescent without, pubescent within; petals ovate, clawed, 3-4 mm. long; stamens numerous; anthers yellow or rarely red. Fruit ovoid or nearly globose, 1.5-2 cm. long, 1-2 cm. broad, bright to dark crimson with pale dots, or orange-yellow with red cheek; flesh yellow, becoming soft and succulent. Stone compressed-ovoid, 10-12 mm. long, 9-10 mm. wide, rounded at base, pointed at apex, slightly keeled and grooved on ventral side.

Growing in thickets, in sandy ground, within the range of the parent species and apparently always in close association with them. Range, from the Arkansas River valley in southeastern Kansas, through central Oklahoma, and probably to be expected also in eastern and central Texas.

NORTH AMERICA. K a n s a s : Arkansas City, Cowley Co., *B. H. Slavin,* no. 164, April 10, July 4, 1914; *E. J. Palmer,* no. 21254, May 11, 1922. O k l a h o m a : Sapulpa, *B. H. Slavin,* no. 132, April 1, June 29, 1914; Muskogee, *B. H. Slavin,* no. 128, March 31, June 26, 1914; Oklahoma City, *B. H. Slavin,* no. 143, July 2, 1914, no. 144, April 4, July 2, 1914, no. 145, April 4, July 2, 1914, no. 146, April 4, July 2, 1914, no. 152, April 5, July 1, 1914 (**type**), no. 252, March 27, June 29, 1916; Norman, *B. H. Slavin,* no. 251, March 27, June 29, 1916; Chickasaw, *B. H. Slavin,* no. 257, March 29, 1916, no. 259, March 29, June 30, 1916, no. 260, June 30, 1916, no. 262, March 29, June 30, 1916; Kingfisher, *B. H. Slavin,* no. 329, April 12, July 5, 1916, no. 330, April 12, July 5, 1916, no. 331, April 12, July 5, 1916, no. 332, April 12, July 5, 1916, no. 334, July 5, 1916; Anadarko, *E. J. Palmer,* no. 12601, July 20, 1917. Also cultivated in the Arnold Arboretum and in Durand-Eastman Park, Rochester, N. Y.

The Chickasaw Plum (*Prunus angustifolia* Marsh.) is widely dis-

tributed in the southern states, from Maryland and Delaware to Florida, Oklahoma, and Texas. In the western part of its range, the var. *varians* Wight & Hedrick, is the commoner form, and it is found abundantly in sandy soil in central Kansas, Oklahoma, and northwestern, central and eastern Texas, where it often forms large thickets of spiny shrubs, 4 to 6 or eight feet in height. The yellow or red fruit matures early and is often of excellent quality. The leaves are prevailingly lanceolate, thin, nearly or quite glabrous, and with only the mid-veins prominent. They are usually conduplicate, making them appear narrower than they really are.

The Sand Plum (*Prunus gracilis* Engelm. & Gray) is found in the western part of the same range, from the valley of the Arkansas River, in southeastern Kansas, and along the western border of southern Arkansas, through most of Oklahoma and eastern Texas as far west as the Brazos River. It is a low slender shrub, usually from 1 to 4 feet in height. The leaves are oval or ovate, gray-green, of firm texture, slightly pubescent above and densely so beneath, and with prominent reticulate veins. The fruit is slightly smaller than that of the Chickasaw Plum, and is edible. It is sometimes borne in such profusion as to weigh the slender branches to the ground. The two species bloom simultaneously and apparently hybridize freely, judging by the number of specimens found.

Prunus Slavinii is quite intermediate in habit and character between the two parent species, and different individuals differ considerably in size and in the pubescence and prominence of the reticulation of the leaves. The specific name is for Mr. B. H. Slavin, superintendent of the splendid park system of Rochester, N. Y., who first collected this interesting Plum and brought it into cultivation there and at the Arnold Arboretum. The type specimen and all of the other numbers cited here are in the herbarium of the Arnold Arboretum.

ERNEST J. PALMER

Calophaca sinica, sp. nov.

Frutex erectus ramis robustis, hornotinis dense albido-pubescentibus, annotinis cortice purpureo-fusco laminis soluto ochraceo-albidis. Stipulae scariosae diu persistentes. Folia pinnata, cum petiolo 3-5 cm. longa, pleraque 7-foliolata; foliola chartacea, ovalia vel obovato-ovalia, 12-18 mm. longa et 7-12 mm. lata, apice rotundata vel truncata, basi rotundata et saepe leviter subcordata, supra cinereo-viridia, maturitate fere glabra, subtus pallidiora, minute et laxe villosula, reticulo venularum satis manifesto, nervis utrinsecus 5-6, supra leviter subtus magis elevatis; petioluli villosi, 1 mm. breviores; petioli 5-12 mm. longi, ut rhachis albido-villosuli. Pedunculi circiter 4 cm. longi longe patentim

villosi et apicem versus stipitato-glandulosi, pauciflori; flores non visi; legumen oblongum, circ. 3 cm. longum, villosum, glandulis longe stipitatis crebris munitum, calyce villoso et stipitato-glanduloso lobis lineari-lanceolatis tubum subaequantibus suffultum.

CHINA. S h a n s i : Chiao-cheng hsien, alt. 3000 ft., *W. Y. Hsia*, no. 1032, May 13, 1929.

The discovery of this new species in Shansi is of interest particularly for the reason that it extends the range of the genus farther to the East into the flora of China.

The genus in its restricted sense (excl. of sect. *Chesneya*) ranges from southern Russia and the Caspian region through Turkestan (*C. wolgarica* Fisch.), Bokhara (*C. grandiflora* Reg.) Tian-shan (*C. wolgarica* var. *tianschanica* [B. Fedtch.] Popov) to Dsungaria (*C. Hovenii* Schrenk) and now extends into northeastern China.

From *C. wolgarica* the new species differs chiefly in the fewer and larger leaflets, the absence of glands from the rhachis and the lower half of the peduncle; from *C. grandiflora* in the fewer leaflets, few-flowered raceme and the stalked glands of the legume and from *C. Hovenii* chiefly in the larger leaflets the spreading pubescence and the presence of stipitate glands on the inflorescence and the legume.

Acer sect. **Macrantha** Pax, emend. **Rehd.**

The section MACRANTHA is one of the most difficult of the sections of *Acer* on account of the rather uniform character of the inflorescence, flowers and fruit and of the variability of the foliage with apparently intermediate forms between the species. The section is here limited as proposed by me in 1911 (in Sargent, Pl. Wilson. I. 92) where I included into this section proposed by Pax in 1886 (in Bot. Jahrb. VII. 244) several species referred by Pax to his sect. INDIVISA, namely *A. sikkimense* Miq., *A. Hookeri* Miq., *A. Davidi* Franch., *A. laxiflorum* Pax and *A. crataegifolium* Sieb. & Zucc., but I excluded *A. pauciflorum* Franch. & Sav. and *A. erosum* Pax which is a synonym of *A. caudatum* Wall. var. *multiserratum* (Maxim.) Rehd. and belongs like *A. pauciflorum* to the sect. SPICATA. Another species erroneously referred to the MACRANTHA by Handel-Mazzetti and by Fang, is *A. Wardii* W. W. Sm. (*A. mirabile* Hand.-Mazz.) which belongs to the sect. SPICATA into the affinity of *A. sinense* Pax; the inflorescence, though typically paniculate, is sometimes reduced to a simple raceme as in the type of *A. mirabile*, but in the bracted and opposite rather long and ascending pedicels and in the flowers it differs from the MACRANTHA section. Besides the seven Chinese species distinguished here, there are eight species in Eastern Asia outside of China (*A. crataegifolium* Maxim.,

A. capillipes Maxim., *A. tegmentosum* Maxim., *A. rufinerve* S. & Z., *A. micranthum* S. & Z., *A. Tschonoskii* Maxim., *A. morrisonense* Hay. and *A. rubescens* Hay.), three in India (*A. sikkimense* Miq., *A. Hookeri* Miq. and *A. pectinatum* Wall.) and one in Eastern North America (*A. pennsylvanicum* L.).

Of the Chinese species *A. Davidi* is the most widely distributed and is found in all provinces of China except in Hopei and Shantung. The other species are of more restricted distribution and each seems to occupy a fairly well defined area. *Acer laxiflorum* Pax is restricted to Szechuan and southeastern Tibet. *Acer taronense* ranges from northern Szechuan through western Yunnan to eastern Tibet and northern Burma. *Acer Forrestii* is found in southwestern Szechuan and western Yunnan. *Acer Maximowiczii* is a northwestern species and extends from southern Shensi and Kansu into northern Szechuan and into Hupeh; a rather distinct form is found in Kweichou. *Acer Grosseri* with var. *Hersii* is found in northern China from Kansu to Hopei and extends south to Hupeh and through Honan to Anhwei. *Acer Metcalfii* is known so far only from Kwangtung and from Hunan, if *A. Davidi* f. *trilobata* is identical with it.

As neither inflorescence, nor flowers or fruits in this section seems to show distinctive and reliable characters, the following key is based exclusively on the leaves which allows identification of both flowering and fruiting specimens.

KEY TO THE CHINESE SPECIES

A. Leaves not lobed, doubly crenate-serrate with obtusish or acutish teeth ..... 1. *A. Davidi*

AA. Leaves more or less lobed.

B. Margin of leaves with 5 or more acute or acuminate teeth to 1 cm.

C. Leaves with rusty pubescence on the veins beneath;

D. Lateral lobes short, acute; flowers red (?always). 2. *A. laxiflorum*

DD. Lateral lobes elongated, acuminate; teeth acuminate to aristate. ..... 3. *A. taronense*

CC. Leaves glabrous beneath except axillary beards in some species.

D. Leaves doubly and sharply serrate with acuminate teeth; middle lobe elongated, the lateral below the middle of the leaf, long acuminate, sometimes short on part of the leaves.

E. Leaves 6-12 cm. long without or occasionally with small basal lobes, finely and closely doubly serrulate; lateral lobes sometimes short and acute, pointing forward. 4. *A. Forrestii*

EE. Leaves 4-8 cm. long, with distinct basal lobes rarely without, incisely doubly serrate or lobulate; lateral lobes always long-acuminate, spreading...5. *A. Maximowiczii*

DD. Leaves unequally or doubly serrate with obtusish mucronulate teeth; middle lobe triangular-ovate; lateral lobes short, acute or short acuminate, rarely long-acuminate. .....6. *A. Grosseri*

BB. Margins of leaves coarsely and irregularly dentate with 2-3, rarely 4, obtuse teeth to 1 cm., lobes acuminate with entire acumen. .....7. *A. Metcalfii*

1. **Acer Davidi** Franchet in Nouv. Arch. Mus. Paris, sér. 2 VIII. 212 (Pl. David. II. 30) (1884).—Pax in Engler, Pflanzenr. IV.-163, p. 36 (1902).—Rehder in Sargent, Trees & Shrubs, I. 167, t. 83 (1905); in Jour. Arnold Arb. VII. 221 (1926); VIII. 163 (1927); IX. 90 (1928).—Fang in Contr. Biol. Lab. Sci. Soc. China, VII. 174 (1932).—Fig. 1.

Acer Davidi var. *glabrescens* Pax in Hooker, Icon. XIX. sub t. 1897 (1889); in Bot. Jahrb. XXIX. 449 (1900); in Engler, Pflanzenr. IV.-163, p. 36 (1902).—Rehder in Sargent, Trees and Shrubs, I. 167 (1905).

Acer Davidi l. *tomentellum* Schwerin in Gartenfl. XLII. 230 (1893).—Pax in Engler, Pflanzenr. IV.-163, p. 36 (1902) "var. α."

Acer Davidi var. *horizontale* Rehder in Sargent, Trees and Shrubs, I. 168 (1905), pro parte, quoad specim. Wilson, no. 1882.—Non Pax.

Acer Cavaleriei Léveillé in Fedde, Rep. Spec. Nov. x. 432 (1911).

Acer laxiflorum var. *integrifolium* Fang in Contrib. Biol. Lab. Sci. Soc. China, VII. 174 (1932).

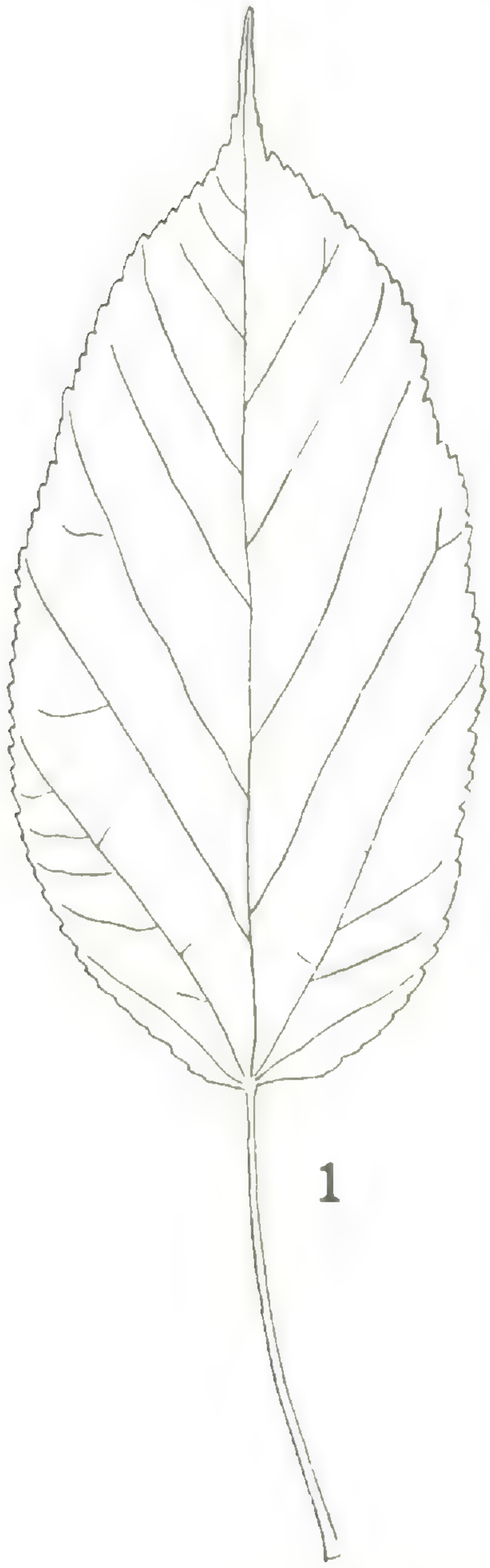
DISTRIBUTION: Kansu, Kiangsi, Chekiang, Anhwei, Kiangsi, Hunan, Kwangtung, Kwangsi, Kweichou, Szechuan, Yunnan and southeastern Tibet.

I have seen numerous specimens from all the provinces of China named above. This shows that the species is widely distributed throughout China except the northern and northeastern provinces Shensi, Shansi, Hopei, Honan and Shantung.

I do not think that var. *glabrescens* Pax is distinct enough to be separated as a variety or form. Its holotype, Henry, no. 7085, shows even on the mature leaves remnants of the brown tomentum. Var. *tomentellum* Schwerin represents the typical form.

Acer Cavaleriei Lévl. of which I have a duplicate of the holotype, Cavalerie, no. 3345, before me differs slightly in the rather narrow oblong leaves 2.8-3.2 cm. wide, rounded, not subcordate at base, quite glabrous beneath, with simple and rather slight crenate serration and an entire acumen; the wings of the fruit spreading horizontally.

Acer laxiflorum var. *integrifolium* Fang from Mt. Omei, Szechuan, of which I have a duplicate of the holotype, Fang, no. 2692, does not seem to differ from *A. Davidi* except that the leaves are comparatively small.



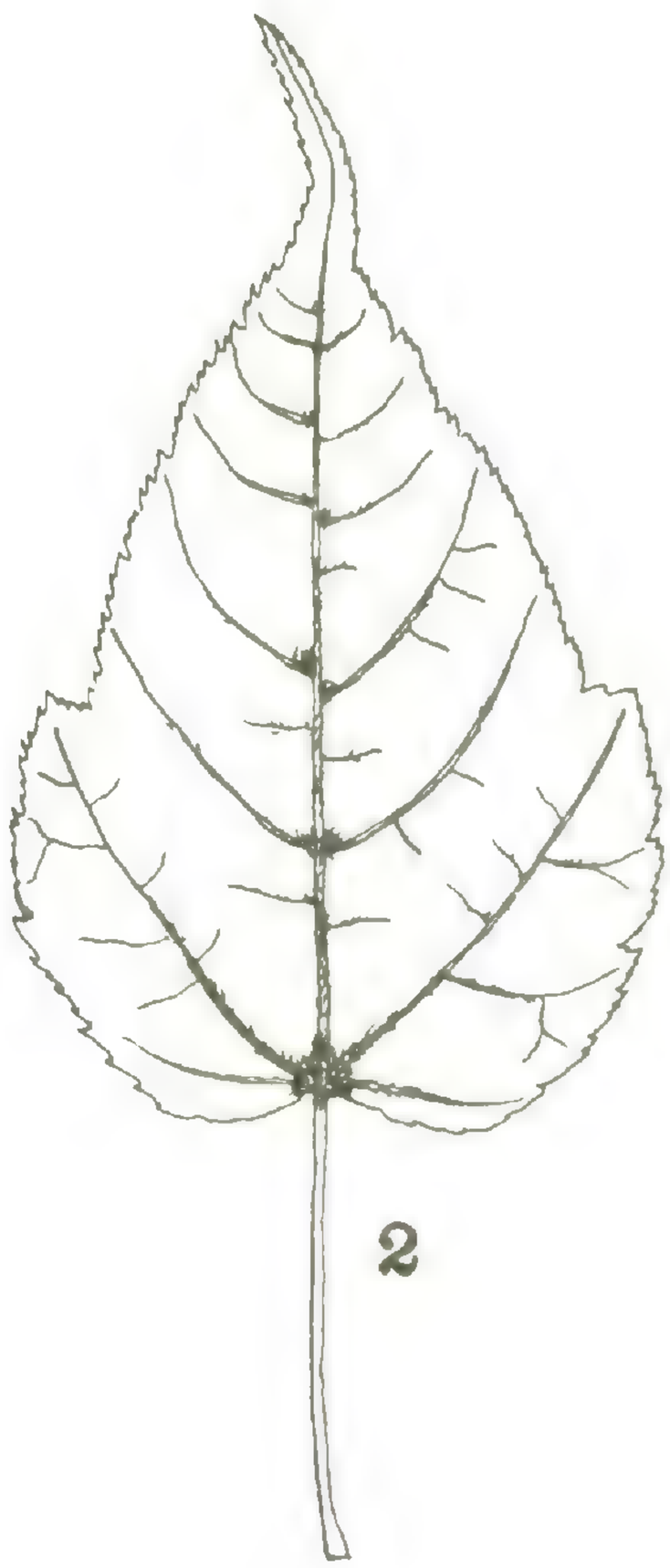


FIG. 1. ACER DAVIDI Franch.: leaf (2/3 nat. size) from Wilson, no. 1005a Mupin (type locality).—FIG. 2. ACER LAXIFLORUM Pax: leaf (2/3 nat. size) from Faber, no. 433, Mt. Omei (syntype).

2. **Acer laxiflorum** Pax in Engler, Pflanzenr. IV.-163, p. 36 (Acer.) (1902).—Rehder in Sargent, Trees & Shrubs, I. 180 (1905); in Sargent, Pl. Wilson. I. 93 (1911), excl. synon.—Fang in Contrib. Biol. Lab. Sci. Soc. China, VII. 178 (1932).—Fig. 2.

SZECHUAN: Mt. Omei, *E. Faber,* no. 453 (syntype), *E. H. Wilson,* Veitch Exp. 3349a, *W. P. Fang,* no. 2874; Nanchuan Hsien, *W. P. Fang,* no. 1191; Pan-han-shan, *E. H. Wilson,* nos. 1904, 4142; Kuan hsien, *W. P. Fang,* no. 2369; Wenchuan-Hsien, *E. H. Wilson,* nos. 1309, 4099, 4108; Wa-shan, *E. H. Wilson,* no. 1154; Mupin, *E. H. Wilson,* nos. 1007, 1007a, 1069, 1234; Tachienlu, *E. H. Wilson,* no. 1309, *W. P. Fang,* no. 3664. SOUTHEASTERN TIBET: Tsarong, *G. Forrest,* no. 21671; Mt. Kenyichunpo and region of Champutong, Salween-Irrawadi watershed, *J. F. Rock,* no. 10242.

Acer laxiflorum is closely related to *A. Davidi* Fr. from which it may be distinguished by the lobed leaves with sharper acute serration, longer and slenderer acumen and with pubescent veins beneath. It also is very close to *A. Forrestii* W. W. Sm. from which it differs in its broader and larger leaves pubescent on the veins beneath though the pubescence is sometimes rather slight. The flowers and young fruits of *A. laxiflorum* are more or less purple or red, but occasionally the flowers may be greenish as in Wilson's no. 1309, though the fruits of the same number are reddish. The flowers of the two other species are always greenish.

Acer laxiflorum var. **longiphyllum** Fang in Contr. Biol. Lab. Sci. Soc. China, VII. 179 (1932).

This variety based on Fang's no. 4513 from Ma-pien-hsien, which I have not seen, does not appear according to the description to differ much, if at all, from the type. Neither have I seen *A. laxiflorum* var. *ningpoense* Pax in Engler, Pflanzenr. IV.-163, p. 36 (1902).

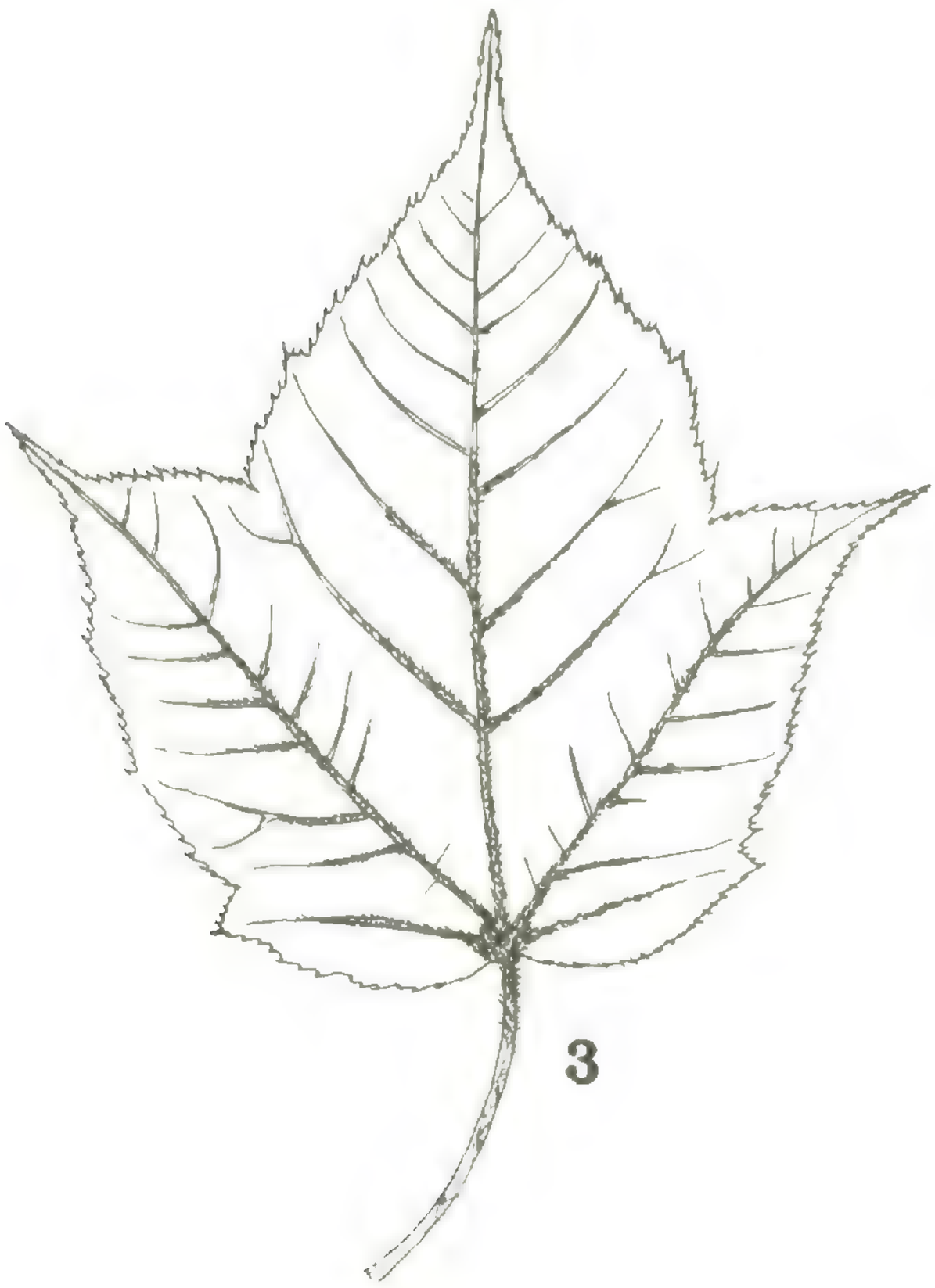
3. **Acer taronense** Handel-Mazzetti in Anz. Akad. Wiss. Wien, 1924, p. 84 (Pl. Nov. Sin. Forts. 25, p. 3).—Fig. 3.

Acer laxiflorum Pax var. *longilobum* Rehder in Sargent, Pl. Wilson. I. 94 (1911), excl. specim. Wilson, 4108.

SZECHUAN: Chin-ting-shan, *E. H. Wilson,* no. 1927 (type of *A. laxiflorum* var. *longilobum*); Tu-ti-liang Mts., Lungan-fu, *E. H. Wilson,* no. 4509. YUNNAN: "prope fines tibeto-birmanicas inter fluvios Ludjiang (Salween) et Djiou-djiang (Irrawaddi), *Handel-Mazzetti,* no. 9385 (type of *A. taronense*); Mt. Gitsa west of Mekong and north of Wei-hsi, *J. F. Rock,* no. 18425; without precise locality, *G. Forrest,* nos. 8990 and 9059. EASTERN TIBET: without precise locality, *G. Forrest,* nos. 26317 and 26581. UPPER BURMA: *G. Forrest,* 26501 and 27269.

Acer taronense is closely related to *A. laxiflorum* Pax, but the leaves differ in the caudate-acuminate lateral lobes, broader and comparatively shorter middle lobe, finer and closer serration with aristate teeth and more densely pubescent veins beneath, though glabrescent at maturity. In the less fine and close serration the Szechuan specimens approach *A. laxiflorum,* but the lateral lobes are caudate-acuminate. On none

of the specimens is the pubescence quite as dense on the veins as in the type specimen. The racemes of the Burma and Tibetan specimens are quite long (about 7-8 cm.) and many-flowered, while in Wilson's no. 1927 they are only 4-5 cm. long and about 10-flowered. It is also closely related to *A. pectinatum* Wall., which is distinguished by the usually 5-lobed leaves more deeply cordate at base and glabrous beneath except the bearded axils; in its very close aristate serration it approaches the specimens of *A. taronense* from Burma.



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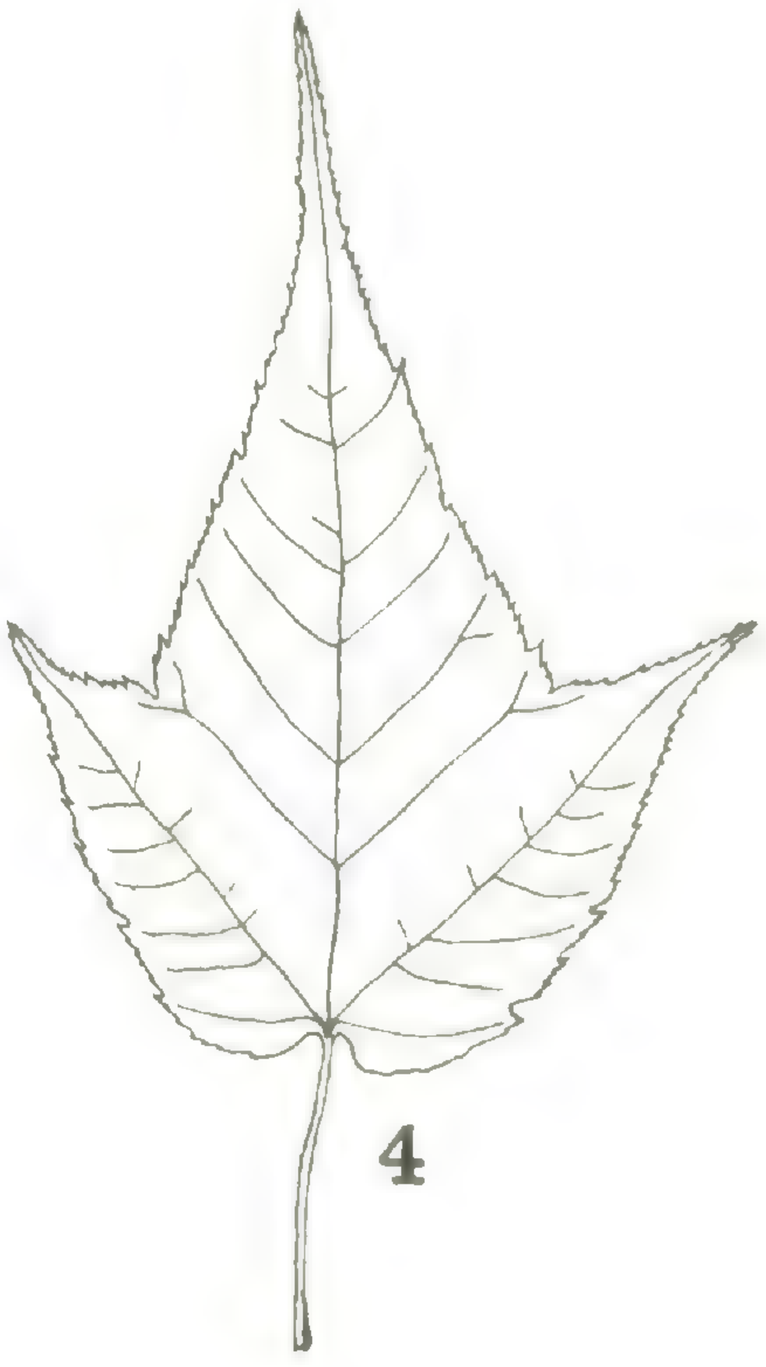

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FIG. 3. ACER TARONENSE Hand.-Mazz.: leaf (2/3 nat. size) from Handel-Mazzetti, no. 9385 (holotype).—FIG. 4. ACER FORRESTII Diels: leaf (2/3 nat. size) from G. Forrest, no. 2106 (holotype).

4. **Acer Forrestii** Diels in Not. Bot. Gard. Edinb. v. 165 (1912).—Fig. 4.

Acer laxiflorum Rehder in Sargent, Pl. Wilson. I. 93 (1911), in part.—Fang in Contr. Biol. Lab. Sci. Soc. China, VII. 178 (1932), in part.—Non Pax.

SOUTHERN SZECHUAN: east of Ning-yuan-fu, *C. Schneider*, no. 959; between Ouentin and Kalapa, *C. Schneider*, no. 1462; between Hunke and Woloho, *C. Schneider*, no. 1499; Kingdom of Muli, *J. F. Rock*, nos.

17993, 18046 and 18230, *G. Forrest,* no. 21337. YUNNAN: Likiang range, *G. Forrest,* no. 2106 (holotype), *C. Schneider,* nos. 1909, 3281, 3338, 3338a, *J. F. Rock,* nos. 3490, 3761, 4231, 5105, 5404; north of Wei-hsi, *J. F. Rock,* no. 17062; near Pe-yen-tsin, *S. Ten,* no. 548; between Chien-chuan plain and Mekong drainage basin, *J. F. Rock,* no. 8630; Chien-chuan-Mekong divide, *G. Forrest,* no. 22380; Mekong-Salween divide, *G. Forrest,* no. 20009, *J. F. Rock,* no. 8893; without precise locality, *G. Forrest,* nos. 10063, 11226, 11279.

Acer Forrestii is closely related to *A. laxiflorum* Pax, but differs in the glabrous and glaucescent under-side of its leaves; the forms with longer acuminate lobes approach *A. Maximowiczii* Pax, but can be distinguished by the finer and closer serration, the absence of the basal pair of lobes and the glaucescent under-side. The flowers are mostly greenish, but Rock's no. 8893 has red flowers; also the fruits of Rock's no. 5404, of Schneider's no. 1909 and Forrest's no. 20009 are distinctly red or reddish.

Acer Forrestii f. **caudatilobum,** forma nova.—Fig. 5.

A typo differt lobis longe caudato-acuminatis, lobis lateralibus lobo medio plerumque plus quam dimidio longioribus.

YUNNAN: Yangtze watershed, western slopes of Likiang Snow Range, *J. F. Rock,* no. 4149, May 30—June 6, 1922 (tree 10 m.; petioles and stems red).

With its caudate-acuminate lobes, the lateral ones mostly more than half as long as the middle lobe, this form looks so strikingly different from the type that it seems desirable to distinguish it as a form.

5. **Acer Maximowiczii** Pax in Hooker, Icon. XIX. sub t. 1897 (1889); in Engler, Pflanzenr. IV.-163, p. 70 (1902).—Rehder in Jour. Arnold Arb. VII. 223 (1926); IX. 90 (1928).—Fang in Contr. Biol. Lab. Sci. Soc. China, VII. 180 (1932).—Fig. 6.

Acer urophyllum Maximowicz in Act. Hort. Petrop. XI. 105 (1890).—Rehder in Sargent, Trees and Shrubs, I. 169, t. 84 (1905).

HUPEH: without precise locality, *A. Henry,* nos. 6857 (syntype) and 6783, *E. H. Wilson,* Veitch Exp. nos. 1891 and 2343; Mt. Ngo-san, *Hugh Scallan;* Chang-yang, *E. H. Wilson,* Veitch Exp. no. 724; South Wushan, *E. H. Wilson,* no. 229; Fang-hsien, *E. H. Wilson,* nos. 355 (in part), 1914 (in part) and 4427; Hsing-shan-hsien, *E. H. Wilson,* nos. 355 (in part) and 1914 (in part); Ichang, *E. H. Wilson,* nos. 355 (in part) and 1914 (in part); Hsao-lung-T'an, *W. Y. Chun,* nos. 4220 and 4618; Shin-tien-tze, *W. Y. Chun,* 4030. SHENSI: Tai-pei-shan, *W. Purdom,* nos. 947 and 948. KANSU: Lower Tebbu country, Want-sang forests, *J. F. Rock,* nos. 14682, 14703, 14706, 14730, 14814, 14855,

15031, 15041 and 15047; Tsaushi-ku, *J. F. Rock*, no. 14735; Dayaya, *J. F. Rock*, no. 14784; Pezhu valley, *J. F. Rock*, no. 14946; Tsaoshiku, *J. F. Rock*, no. 14998; Lien-hoa-shan, Shanshen-miao, *J. F. Rock*, no. 13488; vicinity of Choni, *R. C. Ching*, no. 1009. SZECHUAN: Sing-pan-hsien, *E. H. Wilson*, nos. 4100 and 4513, *W. P. Fang*, no. 4171; Nanchuan-hsien, *W. P. Fang*, no. 931. KWEICHOU: Fan-ching-shan, *Steward, Chiao* & *Cheo*, no. 500.

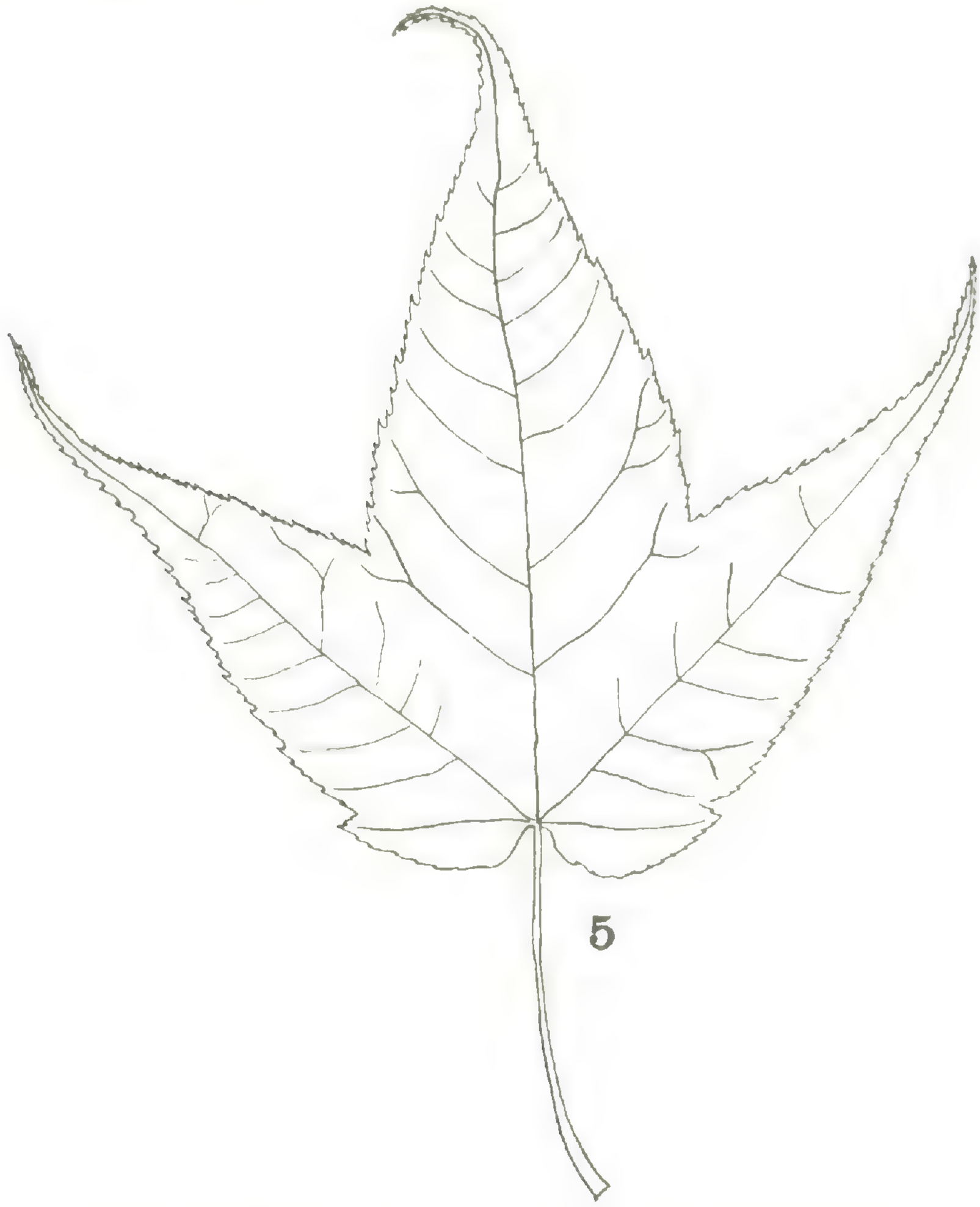


FIG. 5. ACER FORRESTII f. CAUDATILOBUM Rehd.: leaf (2/3 nat. size) from J. F. Rock, no. 4149 (holotype).

Acer Maximowiczii is very similar to *A. Forrestii* Diels, but may be distinguished by the smaller leaves with coarser, distinctly double and even lobulate serration, by the presence of two small basal lobes, though often much reduced or sometimes lacking, and by the more elongated and spreading lateral lobes. In the Kansu specimens collected by Rock the basal lobe is mostly lacking, but the serration and the general

shape and size of the leaf is that of *A. Maximowiczii*. The specimen from Kweichou (Steward, no. 500) has much larger leaves up to 10.5 cm. long and 8.5 cm. wide with the teeth more acuminate, but they are distinctly 5-lobed and lobulate and the lobes abruptly contracted in the long acumen. Possibly it should be considered a distinct variety.

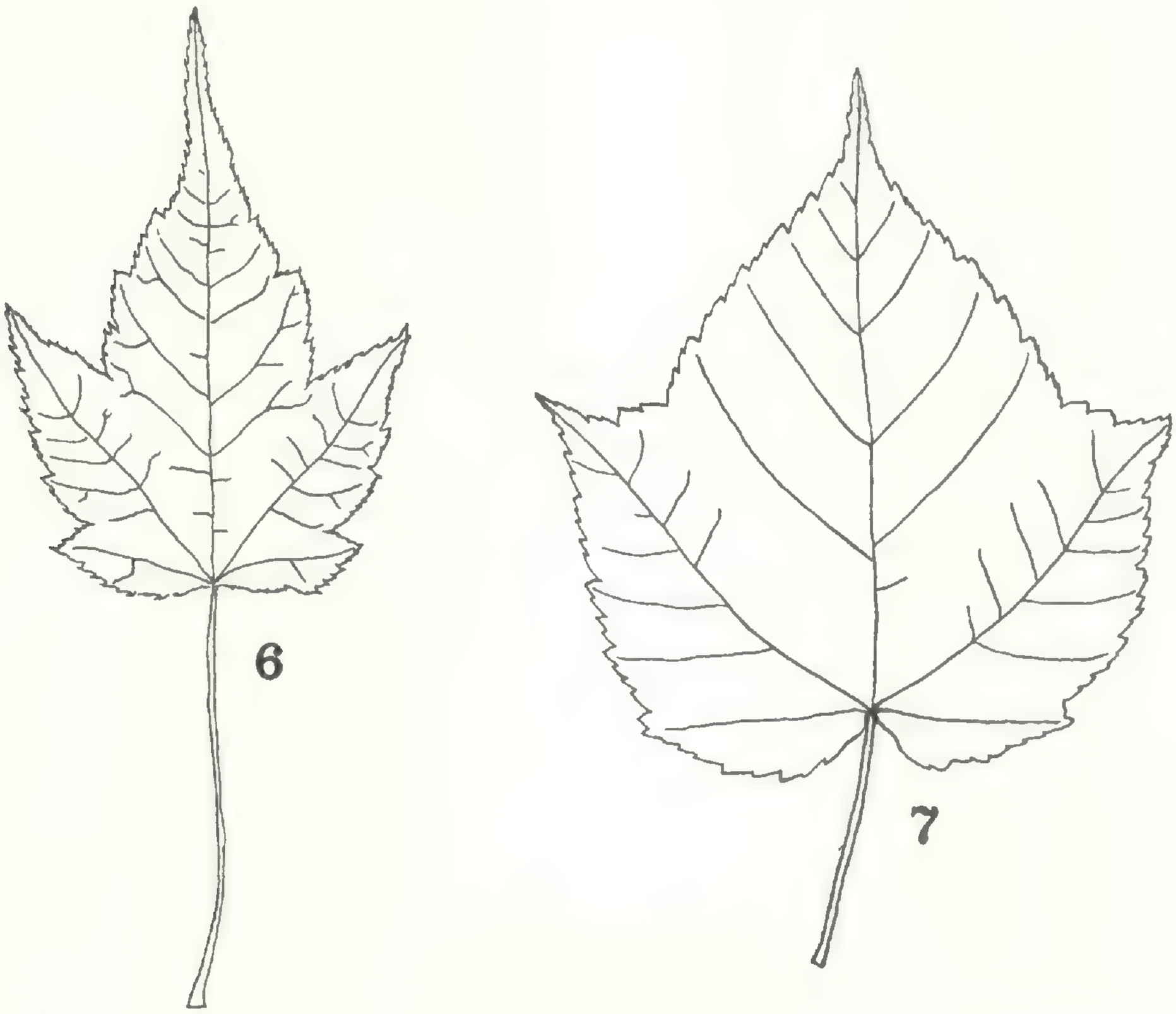


FIG. 6. ACER MAXIMOWICZII Pax: leaf (2/3 nat. size) from Henry, no. 6783. FIG. 7. ACER GROSSERI Pax: leaf (2/3 nat. size) from Harry Smith, no. 7932, Shansi.

Acer Grosseri Pax in Engler, Pflanzenr. IV.-163, p. 80 (1902).—Rehder in Sargent, Trees & Shrubs, I. 181 (1905); in Jour. Arnold Arb. VII. 222 (1926); VIII. 163 (1927); IX. 90 (1928).—Fang in Contr. Biol. Lab. Sci. Soc. China, VII. 181 (1932).—Fig. 7.

Acer Davidii var. γ *horizontalis* Pax in Engler, Pflanzenr. IV.-163, p. 79 (1902); in Bot. Jahrb. XXXVI. beibl. LXXXII. 72 (1905).—Rehder in Sargent, Trees and Shrubs, I. 168 (1905), excl. Wilson. no. 1882.—Hers in Jour. N. China Branch R. As. Soc. LIII. 106 (1922); Liste Ess. Lign. Honan Sept. 1 (1922).

Acer Davidii var. *glabrescens* Pax in Bot. Jahrb. XXXVI. beibl. LXXXII. 73 (1905).—Non Pax (1889).

Acer Pavolinii Pampanini in Nouv. Giorn. Bot. Ital. XVII. 422 (1910).

Acer Hersii Rehder in Jour. Arnold Arb. III. 217 (1922), pro parte.

HOPEI: without precise locality, *Père Chanet,* no. 90. SOUTHERN SHANSI: Chich-hsin, *Harry Smith,* no. 7932, 5895; Shih-li-p'o-shan, *Harry Smith,* no. 6780; Mien-shan, Lin-shih-hsien, *T. Tang,* no. 970; Chin-yuan, Lin-kon-shan, *K. Ling,* no. 9346. SHENSI: Kan-y-san, *G. Giraldi,* no. 2121 (holotype; photo. in A. A.); Lin-tou-san, *G. Giraldi,* July 14, 1897; Mt. Marg-hua-san, west of Singan-fu, *G. Giraldi,* Oct.-Nov. 1894; Tai-pei-shan, *W. Purdom,* no. 949; Thui-kio-tsuen and Mt. Ngo-san, *Hugh Scallan,* in 1899; Hua-shan, *J. Hers,* no. 3080; 60 km. south of Sian-fu, *J. Hers,* nos. 2950, 2999; Lung-chow, *J. Hers,* no. 2359. KANSU: Lower Tebbu country, Mayaku, *J. F. Rock,* no. 15053. HUPEH: Ku-tcen, *C. Silvestri,* no. 1377 (syntype of *A. Pavolinii;* photo. in A. A.); Ou-tan-scian, *C. Silvestri,* nos. 1370, 1371. NORTH HONAN: Sung-shien, *J. Hers,* no. 533; Lu-shi, *J. Hers,* no. 1169. ANHWEI: Chu-hwa-shan, *R. C. Ching,* no. 2613.

Acer Grosseri is very close to *A. Davidi* from which it differs chiefly in its lobed leaves and the somewhat sharper serration, but the lobes sometimes are very short or nearly obsolete which makes it difficult to separate the two species. Such intermediate specimens are e. g. K. Ling, no. 9346, from Shensi, and Giraldi's Mt. Mong-hua-san specimen and Purdom no. 949 from Shensi, but the slightly lobulate margin, the sharper serration and the glabrous under side refer them to *A. Grosseri.*

In the type specimen the middle lobe of the leaf is broadly triangular-ovate, while in other specimens it becomes elongated and oblong-ovate which gives the leaves a resemblance to those of *A. laxiflorum* Pax, but the latter has the leaves pubescent on the veins of the under side and a sharper and deeper serration.

Acer Grosseri var. **Hersii** (Rehd.), comb. nov.—Fig. 8.

Acer Hersii Rehder in Jour. Arnold Arb. III. 217 (1922); VII. 222 (1926); VIII. 163 (1927).—Fang in Contrib. Biol. Lab. Soc. Sci. China, VII. 180 (1932).

Acer sp. allied to *A. Grosseri* Bailey, Gent. Herb. I. 35 (1920).

HONAN: Teng-feng-hsien, *J. Hers,* nos. 219 (holotype) and 2780; Tsi-yuan-hsien, *J. Hers,* nos. 1739 and 2800; Kikung-shan, *A. N. Steward,* no. 9768 (in part) Aug. 3, 1925. HUPEH: Kikungshan, *A. N. Steward,* no. 9768 (in part) July 1925; *L. H. Bailey,* June 16, 1917. ANHWEI: Tsin-tai, Chu-kwa-shan, *R. C. Ching,* no. 2789.

This variety differs from the type in the more elongated long-acuminate lateral lobes, most pronounced in Steward's no. 9768 from the Kikung-shan, in which the lateral lobes are nearly as long as the middle lobe.

Acer Metcalfii, sp. nov.—Fig. 9.

Acer Davidi forma *trilobata* Diels in Notizbl. Bot. Gard. Mus. Berlin, XI. 211 (1931).—Fang in Contr. Biol. Lab. Sci. Soc. China, VII. 177 (1932).

Arbor 10-metralis, glaberrima, ramulis laevibus fuscis vel flavescentibus. Folia decidua, subcoriacea, trilobata, basi subcordata, lobis medio et lateralibus caudato-acuminatis acumine basi except integro, grosse inaequaliter serrato-dentatis dentibus obtusiusculis, nervis lateralibus

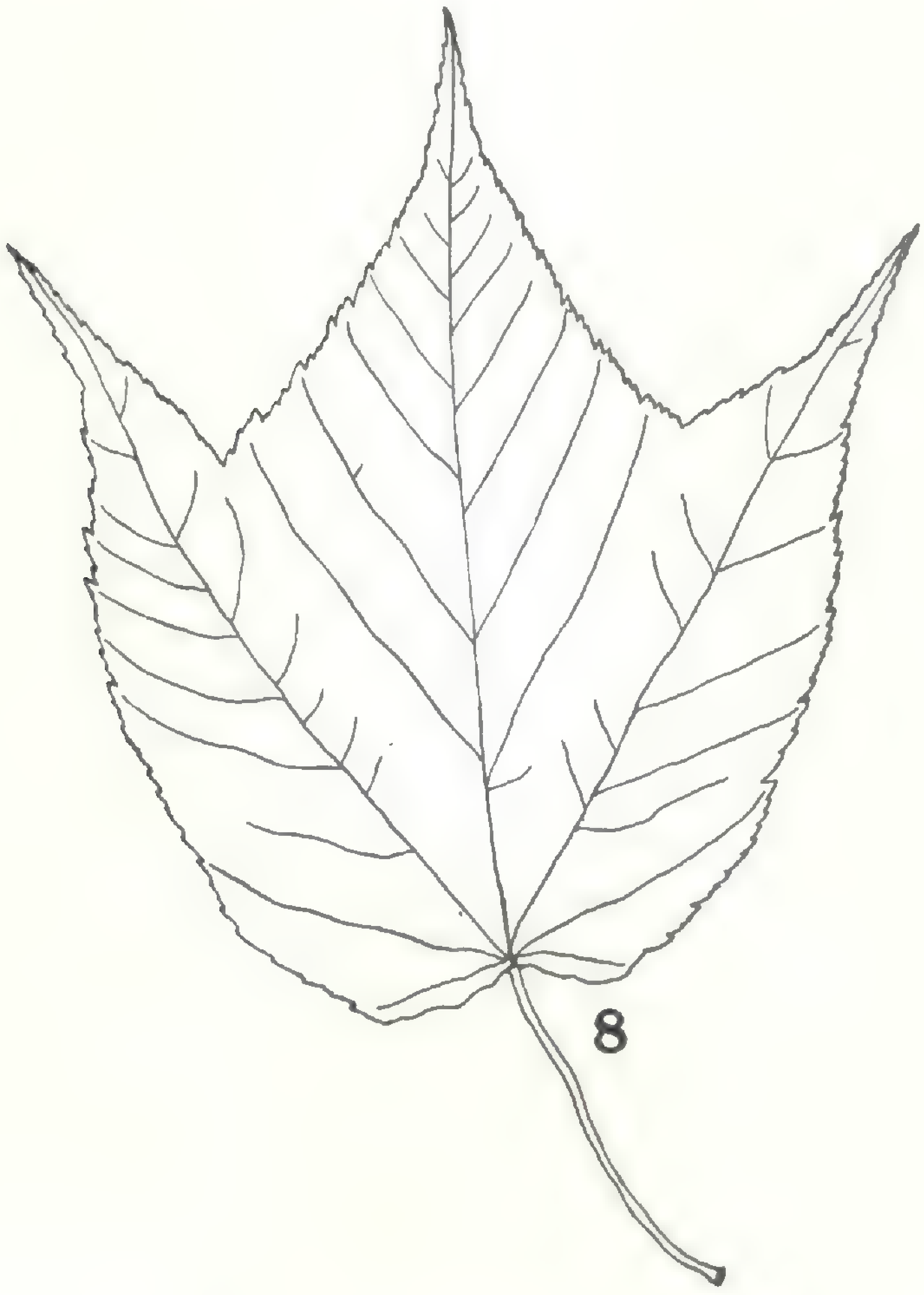


FIG. 8. ACER GROSSERI var. HERSII Rehd.: leaf (2/3 nat. size) from A. N. Steward, no. 9768, Honan.

lobi medii 8-9 in dentes exeuntibus et tantum dentibus duobus vel uno vel nullo inter nervos laterales, utrinque in sicco conspicue reticulata et brunneo-viridia subtus vix pallidiora; petioli circiter 2.5 cm. longi. Flores non visi. Racemi fructiferi 5-6 cm. longi, fructibus 4-6, nuculis compressis nervosis fere horizontalibus circ. 6 mm. longis, alis leviter ascendentibus angulum latum formantibus, nuculo excluso 1.5-2 cm. longis et 6-7 mm. latis.

KWANGTUNG: Lung-tan Mountain, near Iu, Herb. Canton Christ.

Coll. no. 12135, May 22-July 5, 1924 (type). SOUTHERN HUNAN: without precise locality, *S. S. Sin*, no. 298, May to Aug. 1926.

This new species is closely related to *A. Grosseri* var. *Hersii* Rehd. but is easily distinguished by the subcoriaceous leaves reticulate on both sides remotely and coarsely dentate-serrate with obtusish teeth; between the teeth terminating the lateral veins, there are only one or two or rarely three secondary teeth and none at all toward the apex of the lobes. Of *Acer Davidi* f. *trilobata* I have before me only a photograph

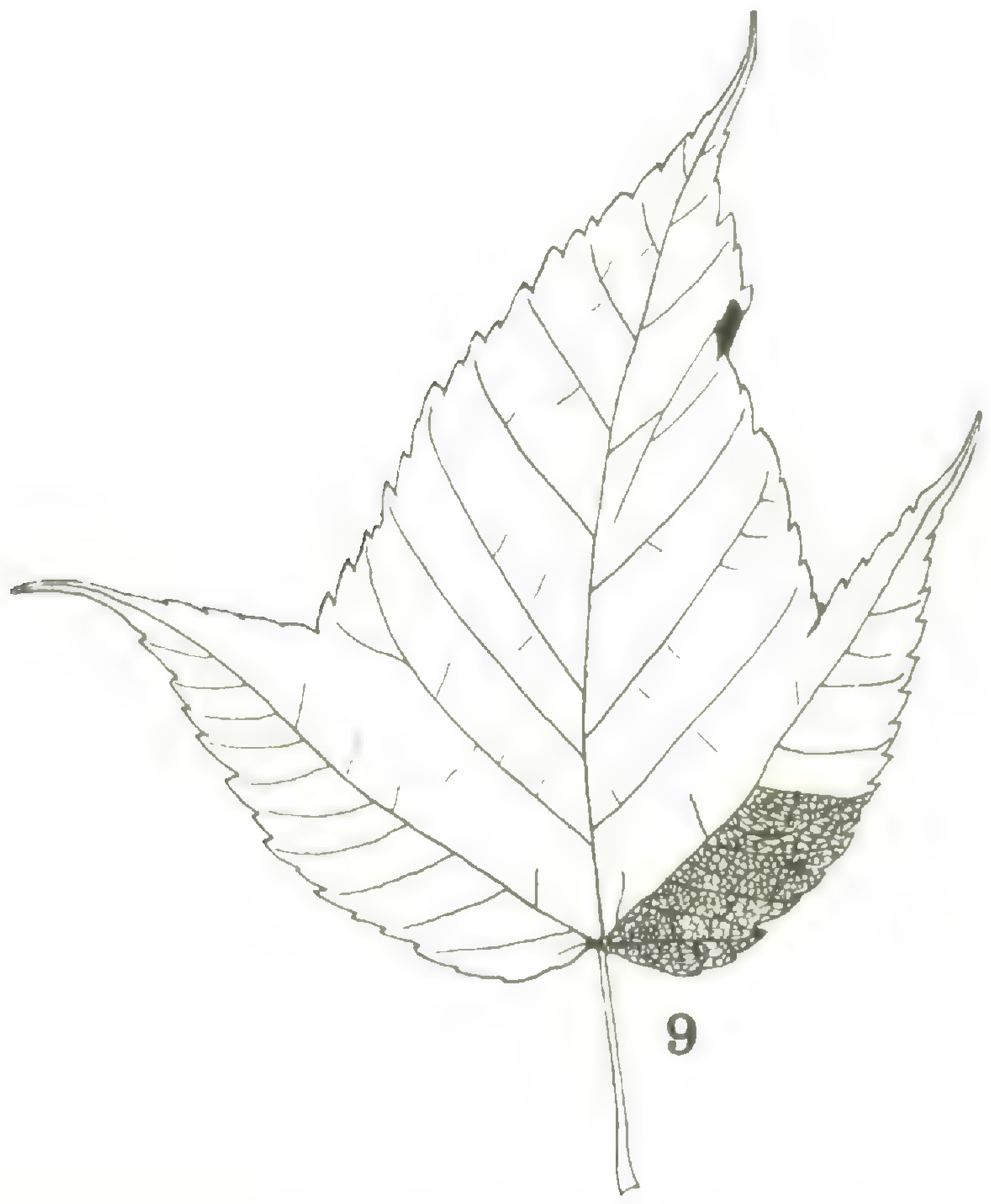


FIG. 9. ACER METCALFII Rehd.: leaf (2/3 nat. size) from Canton Christ. Coll. no. 12135 (holotype).

of the type which shows a serration somewhat less coarse and remote than that of the type of *A. Metcalfii*, but it certainly is referable rather to that species than to *A. Grosseri* var. *Hersii*.

I take pleasure in naming this new species in honor of Dr. F. P. Metcalf, who recognized the specimen as new and marked it thus in this herbarium; his recent paper on the species of the section Integrifolia (Lingnan Sci. Jour. XI. 193-210. 1932) is a valuable contribution to the knowledge of the genus *Acer*.

NOTES ON THE LIGNEOUS PLANTS DESCRIBED BY LEVEILLE FROM EASTERN ASIA[1]

ALFRED REHDER

RUTACEAE

Zanthoxylum simulans Hance in Ann. Sci. Nat. Bot. sér. 5, v. 208 (1866), *"Xanthoxylum."*—Rehder in Jour. Arnold Arb. VII. 181 (1826).

Zanthoxylum Bungei Planchon in Ann. Sci. Nat. Bot. sér. 3, XIX. 82 (1853), nomen.—Hance in Jour. Bot. XIII. 131 (1875) *"Xanthoxylum"; non* Ann. Sci. Nat. sér. v. 209 (1866).

Zanthoxylum Argyi Léveillé in Mem. Acad. Ci. Barcelona, XII. 560 (Cat. Pl. Kiang-Sou, 20) (1916).—**Synon. nov.**

CHINA. K i a n g s u : montagnes, *d'Argy,* May (1846-66) (holotype of *Z. Argyi;* merotype in A. A.).

Zanthoxylum stenophyllum Hemsley in Ann. Bot. IX. 147 (1895).—Rehder & Wilson in Sargent, Pl. Wilson. II. 127 (1914).

Zanthoxylum Esquirolii Léveillé in Fedde, Rep. Spec. Nov. XIII. 266 (1914); Fl. Kouy-Tchéou, 377 (1915).—**Synon. nov.**

CHINA. K w e i c h o u : without locality, *J. Esquirol,* no. 425, June 1904 "arbrisseau" (holotype of *Z. Esquirolii;* photo. and fragments in A. A.).

Zanthoxylum Chaffanjoni Léveillé in Fedde, Rep. Spec. Nov. XIII. 266 (1914).

Zanthoxylum oxyphyllum Léveillé, Fl. Kouy-Tchéou, 377 (1915).—Non Edgeworth.

CHINA. K w e i c h o u : environs de Kouy-yang, mont du Collège, *J. Chaffonjon* in herb. Bodinier, no. 2171, April 12, 1898 "arbuste liane épineux" (holotype; photo. in A. A.).

This species which belongs to the section Fagara D. Don differs from *Z. oxyphyllum* Edgew. to which it was referred by Léveillé in 1916 (l. c.) chiefly in the 1-3 pairs of minutely serrulate leaflets, in the small inflorescences and the small flowers with the perianth only about 3 mm. long. It is rather similar to *Z. cuspidatum* Champ. but easily distinguished by the serrulate fewer leaflets, the smaller inflorescence and the distinct pedicels 1-3 mm. long; from *Z. nitidum* DC. it differs in the acuminate serrulate leaflets.

[1]Continued from vol. XIII. 332; for preceding parts see vol. X. 108-132, 184-196 and vol. XII. 275-281.

Zanthoxylum dissitum Hemsley in Jour. Linn. Soc. XXIII. 106 (1886).—Rehder & Wilson in Sargent, Pl. Wilson. II. 128 (1914).—Léveillé, Fl. Kouy-Tchéou, 377 (1915).

Zanthoxylum Bodinieri Léveillé in Fedde Rep. Spec. Nov. XIII. 266 (1914).

CHINA. K w e i c h o u : environs de Kouy-yang, mont du Collège, trou au pied de la montagne de Ste. Anne (item à Tsin-gay, Che-téou-tchay) *E. Bodinier,* no. 2058 (in part; flower buds), Feb. 10, 1898 "grande liane épineuse," (syntype of *Z. Bodinieri*); environs de Gan-pin, torrent des Ligularia, *L. Martin* & *E. Bodinier,* no. 2058 (in part; open flowers), Feb. 25, 1898 "grande liane épineuse" (syntype of *Z. Bodinieri;* photo in A. A.); Pin-fa, bois, *J. Cavalerie,* no. 748, Dec. 4, 1902 "fruit à odeur forte" (syntype of *Z. Bodinieri;* photo. in A. A.).

Zanthoxylum Bodinieri was enumerated by Léveillé in 1915 as a synonym of *Z. dissitum* Hemsl.

Zanthoxylum odoratum Léveillé in Fedde, Rep. Spec. Nov. XIII. 266 (1914).

Evodia odorata Léveillé in Fedde, Rep. Spec. Nov. IX. 458 (1911); Fl. Kouy-Tchéou, 375 (key) (1915).

Fagara gigantea Handel-Mazzetti in Akad. Anz. Wien, 1921, p. 64 (Pl. Nov. Sin. Forts. 10, p. 2) (1921).—**Synon. nov.**

Zanthoxylum giganteum (Hand.-Mazz.) Rehder in Jour. Arnold Arb. VIII. 151 (1927).

CHINA. K w e i c h o u : Ma-jo, *J. Cavalerie,* no. 2978, Aug. 1908 "odeur forte et agreable" (holotype of *Evodia odorata,* in fruit; photo. in A. A.); Pin-fa, montagne *J. Cavalerie,* no. 1771, April 17, 1904, "trouvé un pied seulement, 3 ou 4 m. de haut; les nombreuses fleurs blan. avaient une odeur forte et agreable"; cited under *Zanthoxylum odoratum;* photo. in A. A.); Ta-hi-yen, Feng-hsiang-ping, on bushy slope, alt. 1700 m., *Steward, Chiao* & *Cheo,* no. 708, Oct. 18, 1931 (tree 8 m. high, 40 cm. diam.; fruit reddish). H u n a n : in monte Yunschan prope urbem Wukang, in silva, alt. 1150-1250 m., *Handel-Mazzetti,* no. 12327, Aug. 8, 1918 "arbor 15 m.; fl. virenti-flavi cum foliis citriodori" (holotype of *Fagara gigantea;* isotype in A. A.).

Under *Evodia odorata* Léveillé enumerates only Cavalerie's no. 2978 and under *Z. odoratum* he enumerates only Cavalerie's no. 1771, though he cites *E. odorata* as synonym. Cavalerie no. 1771 bears inflorescences with flower-buds like Handel-Mazzetti's specimen with which it agrees perfectly; Steward, Chiao and Cheo, no. 708, has ripe fruits.

Orixa japonica Thunberg, Fl. Jap. 61 (1784).—Rehder & Wilson in Sargent, Pl. Wilson. II. 135 (1914).

Sabia Feddei Léveillé in Fedde, Rep. Spec. Nov. IX. 456 (1911); Fl. Kouy-Tchéou, 379 (1915).—**Synon. nov.**

Sabia Cavalerici Léveillé in Fedde, Rep. Spec. Nov. IX. 456 (1911); Fl. Kouy-Tchéou, 378 (1915).—**Synon. nov.**

Glochidion Vanioti Léveillé in Fl. Kouy-Tchéou, 164 (1914).—**Synon. nov.**

CHINA. Kweichou: Pin-fa, *J. Cavalerie,* no. 22bis, April 4, 1902 "2-3m.; bonne odeur; fleurs vertes" (holotype of *Sabia Feddei;* merotype in A. A.); Pin-fa, *J. Cavalerie,* no. 23bis, April 4, 1902 "petal. 5, vert-jaunes" (holotype of *S. Cavaleriei,* photo. in A. A.); Pin-fa, bois de La-tong, *J. Cavalerie,* no. 575, Oct. 1, 1902 "arbuste" (holotype of *Glochidion Vanioti,* photo. in A. A.).

The specimen of *Sabia Feddei* bears staminate and that of *S. Cavaleriei* pistillate flowers, that of *Glochidion Vanioti* is in fruit.

Boenninghausenia albiflora (Hook.) Reichenbach apud Heynhold, Nomencl. Bot. Hort. I. 126 (1840).[1]—Léveillé, Fl. Kouy-Tchéou, 374 (1915).

Bodiniera thalictrifolia Léveillé & Vaniot in Bull. Acad. Intern. Géog. Bot. XI. 48 (1902).

CHINA. Kweichou: mont de Kao-po (Tsin-gay), haies, herbages de la haute montagne, *J. Laborde* in herb. Bodinier, no. 2702, Nov. 8, 1899 "petales d'un blanc pur" (syntype of *Bodiniera thalictrifolia,* photo. in A. A.); environs de Hoang-ko-chou, haies, buissons, *J. Seguin* in herb. Bodinier, no. 2499, Aug. 1898 "fleurs blanches" (syntype of *Bodiniera thalictrifolia,* photo. in A. A.).

Bodiniera thalictrifolia had been already identified with *Boenninghausenia albiflora* as shown by a note on the sheet of Seguin's specimen. If var. *longipes* Franchet, Pl. Delavay. 123 (1889) is to be considered a distinct variety, the specimens cited above should be referred to that variety, but the difference in the length of the stipe of the carpels seems to be too slight and too gradual to maintain var. *longipes* as distinct. In the typical form the stipe is about 3-4 mm. long and the carpels reach to about the middle of the petals, while in var. *longipes* the stipe is about 5-8 mm. and the carpels are nearer the apex of the petals. The typical form occurs in India and Japan, while the var. *longipes* seems to be the prevailing form in China, except in the southwest where the following variety occurs.

Boenninghausenia albiflora var. α **brevipes** Franchet, Pl. Delavay. 123 (1889).

[1]The binomial is usually credited to Reichenbach, Consp. Reg. Veg. 197 (1828), but at the place Reichenbach published neither a description of the genus nor did he transfer the specific epithet of *Ruta albiflora* Hook. which is cited as a synonym. The first generic description was published in 1836 by Meisner, Pl. Vasc. Gen. 60, and the authority for the generic name therefore should be "Reichenbach apud Meisner."

Boenninghausenia sessilicarpa Léveillé in Fedde, Rep. Spec. Nov. XII. 282 (1913).
Boenninghausenia brevipes (Franch.) Léveillé, Cat. Pl. Yun-Nan, 249 (1917).

CHINA. Yunnan: pâturages des mont. derrière Tong-tchouan, alt. 2550-2700 m., *E. E. Maire*, July 1912 "plante vivace, sous-ligneuse, en touffes dressées" (syntype of *B. sessilicarpa;* photo. in A. A.); pied des mont. vallées de Tong-tchouan, alt. 2500 m., *E. E. Maire*, Aug. 1912 "Ancolie sous-ligneuse, rameuse, petites fleurs blanches" (syntype of *B. sessilicarpa;* photo. in A. A.)

This variety is well distinguished from the type by the nearly sessile carpels, but as there are no concomitant characters except that the leaves are rarely thrice pinnate and the leaflets are smaller, it can hardly be considered specifically distinct. It seems, however, geographically well separated; ten specimens from Yunnan and one from southwestern Szechuan close to the Yunnan border have subsessile carpels except one which like the specimens from all other parts of China has long-stipitate carpels.

Toddalia asiatica (L.) Lamarck, Tab. Encycl. Méth. II. 116 (1793).—Rehder & Wilson in Sargent, Pl. Wilson. II. 137 (1914).

Aralia Labordei Léveillé in Bull. Acad. Géog. Bot. XXIV. 144 (1914); Fl. Kouy-Tchéou, 34 (1914).—**Synon. nov.**

CHINA. Kweichou: environs de Tsin-gay, montagne boisée escarpée près de la ville, *J. Laborde*, Nov. 6, 1898 "arbuste" (holotype of *Aralia Labordei;* merotype in A. A.).

Glycosmis Esquirolii (Lévl.) Tanaka in Bull. Soc. Bot. France, LXXV. 709 (1928); in Tanaka Citrus Exp. Sta. I. no. 2, p. 40 (1932).

Clausena Esquirolii Léveillé in Fedde, Rep. Spec. Nov. IX. 324 (1911); Fl. Kouy-Tchéou, 374 (1915).

CHINA. Kweichou: without locality, *J. Esquirol*, no. 744 (holotype of *Clausena Esquirolii*, photo. in A. A.).

Clausena Dunniana Léveillé in Fedde, Rep. Spec. Nov. XI. 67 (1912).

Clausena Willdenowii Léveillé, Fl. Kouy-Tchéou, 375 (1915); non Wight & Arnott.

CHINA. Kweichou: Pin-fa, rochers, *J. Cavalerie*, no. 1072, June 18, 1903 "petit arbrisseau, fleurs blanches" (holotype; photo. in A. A.).

Léveillé reduced this species in his Flore de Kouy-Tchéou to *C. Willdenowii*, but T. Tanaka in revising Léveillé's specimens maintained the name *C. Dunniana*. Also the determinations by Evans of Handel-Mazzetti's no. 10386 from Kweichou as *C. Dunniana* was confirmed by

T. Tanaka according to his note on the specimen in the Arnold Arboretum herbarium.

Citrus ichangensis Swingle in Jour. Agric. Research. I. 1, fig. 1-7, t. 1 (1913); in Sargent, Pl. Wilson. II. 144 (1914).

? Citrus Cavaleriei Léveillé apud Cavalerie in Bull. Géog. Bot. XXI. 211, 236 (1911), nomen.—Koidzumi, Fl. Symb. Or.-As. 55 (1930).

CHINA. K w e i c h o u : (no specimen in herb. Léveillé).

There are no specimens of *Citrus Cavaleriei* in the Léveillé herbarium and the name was published without description by Cavalerie who states that it is a spiny Orange growing wild at an altitude of 1700 m. near Ma-jo and Kai-tchéou in the province of Kweichou. In the same article Cavalerie publishes a few other manuscript names of *Citrus* given by Léveillé. Léveillé states on p. 236 that W. T. Swingle is inclined to refer *C. Cavaleriei* tentatively to *C. hystrix* DC. Koidzumi, however, identifies it (l. c.) with *C. ichangensis* and makes the latter name a synonym of the former, in spite of the fact that *C. Cavaleriei* is a nomen nudum.

SIMAROUBACEAE

Ailanthus Esquirolii Léveillé, Fl. Kouy-Tchéou, 404 (Sept. 1915), nomen; in Monde Pl. sér. 2, XVII. 23 (Nov. 1915).

CHINA. K w e i c h o u : without locality, *J. Cavalerie,* no. 773 (not now in herb. Léveillé).

Léveillé remarks in Monde des plantes (l. c.) that unfortunately the specimen which had been put aside for a future diagnosis has been mislaid and that only a short diagnosis could be given which runs as follows: "differt ab *A. glutinosa* [*sic*] foliis majoribus conspicue dentatis et floribus coloratis." The plant may not be an Ailanthus at all.

MELIACEAE

Chickrassia tabularis A. Jussieu in Mém. Mus. Paris, XIX. 251 (1830).

Dysoxylon Esquirolii Léveillé in Cat. Pl. Yun-Nan, 176 (1916).—**Synon. nov.**

CHINA. K w e i c h o u : Ycoca-may, *J. Esquirol,* no. 858 (? 898) June 1906 "grand arbre, fleurs jaunes" (holotype of *Dysoxylon Esquirolii;* merotype in A. A.).

The specimen represents a form with the leaves soft-pubescent beneath and sparingly pubescent above; also the rhachis of the leaf and the inflorescence is finely pubescent.

Cipadessa baccifera Miq. var. **sinensis** Rehder & Wilson in Sargent, Pl. Wilson. II. 159 (1914).

Rhus Blinii Léveillé, Fl. Kouy-Tchéou, 411 (1915).—**Synon. nov.**

CHINA. K w e i c h o u : sud de Tin-fan, *J. Cavalerie,* no. 1911, Nov. 1904 (holotype of *Rhus Blinii;* photo. in A. A.).

MALPIGHIACEAE

Aspidopterys Cavaleriei Léveillé in Fedde, Rep. Spec. Nov. IX. 458 (1911); Fl. Kouy-Tchéou, 271 (1914) in part.—Hutchinson in Kew Bull. Misc. Inform. 1917, p. 97.—Niedenzu in Engler, Pflanzenr. IV.-141 (Heft 91) p. 32 (Malpigh.) (1928).

Aspidopterys Dunniana Léveillé in Fedde, Rep. Spec. Nov. XI. 65 (1912); Fl. Kouy-Tchéou, 271 (1914).—Niedenzu, l. c. (1928).

CHINA. K w e i c h o u : Lo-fou, *J. Cavalerie,* no. 2993, April 1908 (holotype of *A. Cavaleriei* and syntype of *A. Dunniana;* photo. in A. A.); Lo-fou, *J. Cavalerie,* no. 3477, March 1909 "fleurs blanches" (syntype of *A. Dunniana;* photo. and fragments in A. A.).

Aspidopterys Dunniana was referred as a synonym to *A. Cavaleriei* by Hutchinson who drew attention to the fact that Léveillé cites Cavalerie no. 2993 under both species, under *A. Dunniana* together with Cavalerie, no. 3477. In his Flore de Kouy-Tchéou, however, Léveillé omits Cavalerie's no. 2993 under *A. Cavaleriei* and cites instead Cavalerie, no. 1882, and Esquirol, no. 712, which do not belong to *Aspidopterys* at all, but represent Combretaceae.

Aspidopterys Esquirolii Léveillé in Fedde, Rep. Spec. Nov. XI. 65 (1912); Fl. Kouy-Tchéou, 271 (1914).—Hutchinson in Kew Bull. Misc. Inform. 1917, p. 100.—Niedenzu in Engler, Pflanzenr. IV.-141 (Heft 91) p. 21 (Malpigh.) (1928).

Cavalierella cordata Léveillé, Fl. Kouy-Tchéou, 61 (1914).—**Synon. nov.**

CHINA. K w e i c h o u : without locality, *J. Esquirol,* no. 593, Aug. 1905 "arbrisseau, fleur jaune" (ex Léveillé et Hutchinson; syntype); Houa-kiang, *J. Cavalerie,* no. 2032, June 6, 1904 (syntype; photo. in A. A.); Gan-chouen, *J. Cavalerie,* no. 3961, May 1912 (holotype of *Cavalierella cordata;* photo. and merotype in A. A.).

This species is according to Hutchinson easily distinguished from the other species by the externally densely hairy sepals.

Esquirol's no. 593 I have not seen, but it is cited by Hutchinson and apparently does not differ from Cavalerie's 3961.

Cavalierella cordata Lévl. one of the two species on which Léveillé based the new genus *Cavalierella* placed into the Caprifoliaceae is undoubtedly an *Aspidopterys* and seems referable to *A. Esquirolii* except that the leaves are subcordate to cordate at the base and generally broader and larger than in Cavalerie's no. 3961; the specimen is in fruit and the fruit is suborbicular, about 3 cm. broad and slightly longer,

cristate between the wings which are furnished with setose hairs except near the margin. The other species of *Cavalierella, C. Dunniana,* belongs to *Dipelta.*

EUPHORBIACEAE

Andrachne Bodinieri Léveillé in Fedde, Rep. Spec. Nov. XII. 187 (1913); Fl. Kouy-Tchéou, 158 (1914).

Andrachne hypoglauca Léveillé, l. c. (1913); l. c. (1914).

CHINA. Kweichou: montagnes de Lou-tsong-koan, rocailles, talus pierreux, *E. Bodinier,* no. 1662, July 12, 1897 "petit arbuste" (holotype of *A. Bodinieri,* merotype in A. A.); bord de la plaine de Tou-chan, *J. Cavalerie,* July 16, 1897 (holotype of *A. hypoglauca;* photo. in A. A.).

This species seems nearest to *A. chinensis* Bge., but differs in its narrower, oblong to lanceolate glabrous leaves, cuneate at the base, with revolute margin and of chartaceous texture with the midrib and lateral veins impressed above and prominent beneath. In the type of *A. hypoglauca* the leaves are narrower and more glaucous beneath than in *A. Bodinieri.* In the shape of the leaves it resembles *A. lolonum* Hand.-Mazz., but this has the leaves densely pubescent beneath.

This and the other species of *Andrachne* described by Léveillé are only mentioned by name as species dubiae by Pax and Hoffmann, in Engler, Pflanzenr. IV.-147, XXV. p. 178 (1922).

Andrachne Esquirolii Léveillé in Fedde, Rep. Spec. Nov. IX. 327 (1911); Fl. Kouy-Tchéou, 158 (1914).

Andrachne persicariifolia Léveillé in Fedde, Rep. Spec. Nov. XI. 187 (1913).

Andrachne attenuata Handel-Mazzetti in Akad. Anz. Wiss. Wien, 1921, p. 178 (Pl. Nov. Sin. Forts. 13, p. 2) (1921). **Synon. nov.**

CHINA. Kweichou: without locality, *J. Esquirol,* no. 110 (holotype of *A. Esquirolii;* photo. in A. A.); environs de Kouy-yang; bois de la pagoda de Kien-lin-chan, dans les rocailles, "item au Ke-ma-tong," *E. Bodinier,* no. 1695, July 20, 1897 (holotype of *A. persicariifolia,* photo. in A. A.); Djitschangping prope oppidum Muyu ad austr.-occ. oppidi Dschenning, *Handel-Mazzetti,* no. 10402, June 22, 1917 (holotype of *A. attenuata;* isotype in A. A.).

In the shape of its leaves and other characters *A. Esquirolii* resembles closely the type of *A. attenuata,* while *A. persicariifolia* has much narrower leaves varying from oblong-lanceolate to narrow-lanceolate, but in the acute sepals and the strigose-setose ovary it agrees with *A. attenuata.*

Securinega suffruticosa (Pall.) Rehder in Jour. Arnold Arb. XIII. 338 (1932).

Securinega ramiflora (Ait.) Mueller Arg. in De Candolle, Prodr. XV. pt. I. 449 (1866).
Securinega fluggeoides Mueller Arg., l. c. 550 (1866).
Phyllanthus Argyi Léveillé in Mem. Acad. Ci. Barcelona, XII. 550 (Cat. Pl. Kiang-Sou, 10) (1916).—**Synon. nov.**

CHINA. Kiangsu: without locality, *d'Argy*, no. 78, (1846-66) "arbrisseau" (holotype of *Phyllanthus Argyi;* merotype in A. A.)

Phyllanthus emblica Linnaeus, Spec. Pl. 982 (1753).
Phyllanthus Mairei Léveillé in Bull. Géog. Bot. XXV. 23 (1915); Cat. Pl. Yun-Nan, 97 (1916).—**Synon. nov.**

CHINA. Yunnan: rives du fleuve Bleu, à Siao-ho, alt. 400 M., *E. E. Maire*, May 1912 "arbrisseau toujours vert; fleurs jaunes; fruits verts, acides, en forme de cerise" (holotype of *P. Mairei*, merotype in A. A.).

Phyllanthus Mairei has been already identified by W. W. Smith with *P. emblica* according to a note on the sheet.

Phyllanthus Franchetiana Léveillé in Bull. Géog. Bot. XXV. 23 (1915); Cat. Pl. Yun-Nan, 97 (1916).

CHINA. Yunnan: river du fleuve Bleu, à Siao-ho, alt. 400 m., *E. E. Maire*, May 1912 "fleurs rougeâtres, drupes vertes, acidulées, en forme de cerise" (holotype; photo. and fragments in A. A.).

This species resembles *P. pulcher* Wall., but the leaves which are 6-8 mm. long are not mucronate and the sepals are crenate-dentate rather than laciniate. The fruits which are not present on the specimen are cherry-like according to the collector's note.

Phyllanthus Dunnianus (Lévl.) Handel-Mazzetti in herb.
Phyllanthodendron Dunnianum Léveillé in Fedde, Rep. Spec. Nov. IX. 324 (1911); Fl. Kouy-Tchéou, 166 (1914).—Handel-Mazzetti, Symb. Sin. VII. 224 (1931).
Phyllanthodendron Cavalerici Léveillé in Fedde, Rep. Spec. Nov. IX. 454 (1911); Fl. Kouy-Tchéou, 116 (1914).—**Synon. nov.**
Phyllanthodendron Dunnianum Lévl. var. *hypoglaucum* Léveillé, Fl. Kouy-Tchéou, 166 (1914).

CHINA. Kweichou: Lo-fou, *J. Cavalerie*, no. 2659, Nov. 1905 "fl. vertes" (holotype of *Phyllanthodendron Dunnianum;* merotype in A. A.); O-to près Lo-fou, *J. Cavalerie*, no. 3284, April 1907 "arbrisseau" (holotype of *Ph. Cavaleriei;* merotype in A. A.); Lo-fou, *J. Cavalerie*, no. 3500, April, May 1909 (holotype of *Ph. Dunnianum* var. *hypoglaucum;* merotype in A. A.).

The three specimens cited above agree in the distinctly winged branchlets and generally in the shape of the leaves, but differ in several other characters. In *Phyllanthodendron Dunnianum* var. *hypoglaucum* (Cavalerie, no. 3500) which is in fruit the leaves are glaucous beneath and the branchlets are pilose, while in the other two specimens they are

perfectly glabrous. *Phyllanthodendron Dunnianum* (Cavalerie, no. 2659) has thin membraneous leaves, while *Ph. Cavaleriei* (Cavalerie, no. 3284) has subcoriaceous leaves with prominent veins on both sides; both specimens are in flower. Cavalerie's nos. 3284 and 3500 have dimorphic leaves, while no. 2659 which consists only of two small sterile branchlets (with a detached flower in a pocket) has only one kind of leaves. The specimen of no. 2659 bears in Handel-Mazzetti's handwriting the new combination "*Phyllanthus Dunnianus* (Lévl.)" but in his remarks on *Phyllanthodendron Dunnianum* under his *Phyllanthus anthopotamicus* (in Symb. Sin. VII. 224. 1931) he does not cite this combination; he only states that the two species are closely related. *Phyllanthus Dunnianus,* however, differs markedly from *P. anthopotamicus* in the strongly angular, slightly winged glabrous or only slightly pilose branchlets, the quite glabrous, more acuminate leaves dark green above and generally larger, and in the larger, longer-stalked glabrous flowers.

Phyllanthus spec.

Sterculia Bodinieri Léveillé, Fl. Kouy-Tchéou, 406 (1915).—**Synon. nov.**

CHINA. K w e i c h o u : environs de Hoang-ko-chou, grande cascade, au bord de l'eau, *J. Seguin* in herb. Bodinier, no. 2194, April 1898 "arbuste, fleurs rougeâtres" (holotype of *Sterculia Bodinieri;* merotype in A. A.).

This plant is undoubtedly a Euphorbiacea and seems referable to the sect. Eriococcus of *Phyllanthus* except that it has 4 anthers.

Glochidion puberum (L.) Hutchinson in Sargent, Pl. Wilson. II. 518 (1916).

Glochidion Bodinieri Léveillé in Fedde, Rep. Spec. Nov. XII. 183 (1913); Fl. Kouy-Tchéou, 163 (1914).—**Synon. nov.**

CHINA. K w e i c h o u : Kouy-yang, mont du Collège, *E. Bodinier,* no. 2307, July 21, 1897 (fruit), June 9, 1898 "arbuste de 1 m., très branchu" (holotype of *G. Bodinieri;* merotype in A. A.).

Glochidion villicaule Hooker f., Fl. Brit. Ind. v. 326 (1887).

Glochidion Esquirolii Léveillé in Fedde, Rep. Spec. Nov. XII. 186 (1913); Fl. Kouy-Tchéou, 163 (1914).—**Synon. nov.**

CHINA. K w e i c h o u : Ouang-mou, *J. Esquirol,* no. 714, June 1904 "fleurs jaunes" (holotype of *G. Esquirolii;* photo. in A. A.).

Baccaurea Cavaleriei Léveillé, Fl. Kouy-Tchéou, 159 (1914).

CHINA. K w e i c h o u : Lo-fou, *J. Cavalerie,* no. 3299, April 1907 "petit arbre" (holotype, photo. in A. A.).

In the general habit and in the leaf this species has some resemblance to *B. sapida* Muell. Arg., but the material is insufficient for exact

determination; it consists of a leafy branch and broken fragments of immature inflorescences.

Antidesma microphyllum Hemsley in Jour. Linn. Soc. Bot. XXVI. 432 (1894).—Handel-Mazzetti, Symb. Sin. VII. 218 (1931).

Antidesma Seguini Léveillé in Fedde, Rep. Spec. Nov. IX. 460 (1911); Fl. Kouy-Tchéou, 158 (1914).—Pax & Hoffmann in Engler, Pflanzenr. IV.-147, XV. 166 (1922).

Myrica Darrisii Léveillé in Fedde, Rep. Spec. Nov. XII. 537 (1913); Fl. Kouy-Tchéou, 281 (1914).—**Synon. nov.**

CHINA. K w e i c h o u : district de Tchen-lin, bord du fleuve à la cascade de Hoang-ko-chan, *J. Seguin,* June 10, 1898 "fleur blanche" (ex Léveillé; syntype of *A. Seguini*); fleuve Hoa-kiang, *J. Esquirol,* no. 505, June 1905; (ex Léveillé; syntype of *A. Seguini*); Tchai-chouị-ho, *J. Esquirol,* no. 1586, July 1909 (syntype of *A. Seguini;* merotype in A. A.); route de Mou-you-se à Houang-ko-chou, *J. Cavalerie,* no. 2058, June 10, 1904 (holotype of *Myrica Darrisii;* photo. and fragment in A. A.).

Of the three syntypes of *A. Seguini* I have before me only Esquirol, no. 1568, which agrees exactly with Henry's 9530a of *A. microphyllum* Hemsl., while Cavalerie, no. 2058, the holotype of *Myrica Darrisii,* differs slightly in, at least partly, somewhat broader leaves. *Antidesma Seguini* had been identified with *A. microphyllum* by Dr. Stapf according to a note in a letter from Dr. Handel-Mazzetti of Dec. 25, 1922.

Croton Tiglium Linnaeus, Spec. Pl. 1004 (1753).—Handel-Mazzetti, Symb. Sin. VII. 218 (1931).

Alchornea Vanioti Léveillé, Cat. Pl. Yun-Nan, 95 (1916).

CHINA. Y u n n a n : Tong-tchouan, *E. E. Maire,* 1911 (holotype of *Alchornea Vanioti;* merotype in A. A.).

Alchornea Vanioti was identified with *Croton Tiglium* by Handel-Mazzetti (l. c.).

Speranskia cantonensis (Hance) Pax & Hoffmann in Engler, Pflanzenr. IV.-147, VI. 15 (Euphorb.) (1912).

Mercurialis acanthocarpa Léveillé in Fedde, Rep. Spec. Nov. III. 21 (1906).

Speranskia tonkinensis Léveillé, Fl. Kouy-Tchéou 167 (1914).

CHINA. K w e i c h o u : Pin-fa, près d'une rivière, très rare, *J. Cavalerie,* no. 1585, Oct. 19, 1903 (holotype of *Mercurialis acanthocarpa;* photo. in A. A.).

Mercurialis acanthocarpa was referred by Léveillé together with *Speranskia Henryi* Oliv., to *Speranskia tonkinensis,* a name cited without author and probably a mistake for *S. cantonensis.*

Mallotus Leveillanus Fedde, Rep. Spec. Nov. X. 144 (1912).—Pax & Hoffmann in Engler, Pflanzenr. IV.-147, VII. p. 165 (1914).

Mallotus Esquirolii Léveillé in Fedde, Rep. Spec. Nov. IX. 461 (1911), non Léveillé, l. c. 327.
Mallotus Leveillei Fedde apud Léveillé, Fl. Kouy-Tchéou, 165 (1914).

CHINA. Kweichou: Ouang-mou, *J. Esquirol,* no. 120, June 1904 (holotype of *M. Esquirolii;* photo. in A. A.); Lo-fou, *J. Cavalerie,* no. 3666, Aug. 1909 (duplicate in A. A.).

Cavalerie's no. 3666 is cited together with Esquirol's no. 120 by Léveillé under *M. Leveillei* (l. c.) and by Pax & Hoffmann under *M. Leveillanus* (l. c.).

Mallotus philippinensis (Lam.) Mueller Arg. in Linnaea, XXXIV. 196 (1865).—Pax & Hoffmann in Engler, Pflanzenr. IV.-147, VII. p. 184 (1914).

Evonymus hypoleucus Léveillé in Fedde, Rep. Spec. Nov. XIII. 260 (1914).—**Synon. nov.**
Phyllanthodendron sp. Léveillé, Fl. Kouy-Tchéou, 166 (1914).

CHINA. Kweichou: Lo-fou, *J. Cavalerie,* no. 2733, April 1906 (holotype of *Evonymus hypoleucus* and *Phyllanthodendron* sp.; photo. in A. A.).

Evonymus hypoleucus is cited by Léveillé in his Flore de Kouy-tchéou as a synonym of *Phyllanthodendron* sp.

Mallotus Esquirolii Léveillé in Fedde, Rep. Spec. Nov. IX. 327 (1911); Fl. Kouy-Tchéou, 165 (1914).—Pax & Hoffmann in Engler Pflanzenr. IV.-147, VII. 196 (1914).

CHINA. Kweichou: without precise locality, *J. Esquirol,* no. 898 (holotype; photo. and fragments in A. A.); Lo-fou, *J. Cavalerie,* no. 3114, March 1909 (cited in Fl. Kouy-Tchéou; duplicate in A. A.).

Mallotus Milliettii Léveillé, Fl. Kouy-Tchéou, 165 (1914).

CHINA. Kweichou: route de Gan-chouen à Hin-y-fou, *Cavalerie,* no. 3967, July 1912 (holotype; photo. and fragments in A. A.).

This species seems nearest to *M. contubernalis* Hance, but differs chiefly in the leaves being sparingly stellate-pubescent above and rather densely and softly so below, and in the subsessile larger capsules about 1.4 cm. in diam. with a heavier indumentum. In the pubescence of the leaves it seems to approach var. *chrysocarpus* (Pamp.) Hand.-Mazz. which I have not seen, the type of it being missing from the Herb. Biondi when I was in Florence in 1932.

Léveillé cites Cavalerie, no. 3697, as the type of his species, but the specimen is numbered 3967 and bears in Léveillé's handwriting the name *Mallotus Cavaleriei* in ink and below in pencil *M. Milliettii.* Léveillé apparently changed the name *M. Cavaleriei* which was never published for this plant, to *M. Milliettii,* because he had already given

the former name to a species which was later referred to *Discocleidion rufescens* (Fr.) Pax & Hoffm.

Discocleidion rufescens (Franch.) Pax & Hoffmann in Engler, Pflanzenr. IV.-147, VII. p. 45, fig. 6 (1914).—Léveillé, Fl. Kouy-Tchéou, 161 (1914).

Mallotus Cavaleriei Léveillé in Fedde, Rep. Spec. Nov. XI. 296 (1912).

CHINA. Kweichou: Gan-chouen, *J. Cavalerie,* no. 3825, June 1910 (holotype of *Mallotus Cavaleriei;* photo. in A. A.).

Acalypha Mairei (Lévl.) Schneider in Sargent, Pl. Wilson. III. 301 (1916).—Léveillé, Cat. Pl. Yun-Nan, 94 (1916).—Pax & Hoffmann in Engler, Pflanzenr. IV.-147, XVI. 137 (1924).

Morus Mairei Léveillé in Fedde, Rep. Spec. Nov. XIII. 265 (1914).

CHINA. Yun-nan: brousse derrière Mo-tsou fleuve Bleu, alt. 800 m., *E. E. Maire,* May (ex Léveillé; syntype of *Morus Mairei*); rochers de Ma-hong, alt. 3000 m., *E. E. Maire,* June 1912 (syntype of *Morus Mairei,* merotype in A. A.).

Tragia involucrata Linnaeus, Spec. Pl. 980 (1753).—Pax & Hoffmann in Engler, Pflanzenr. IV.-147, IX-XI. 81 (1919).—Handel-Mazzetti, Symb. Sin. VII. 218 (1931).

Alchornea Mairei Léveillé, Cat. Pl. Yun-Nan, 94 (1916).

CHINA. Yunnan: vallon de Yon-fong-keou, alt. 800 m., *E. E. Maire,* July 1912 (holotype of *Alchornea Mairei;* photo. in A. A.).

Alchornea Mairei was first identified with *Tragia involucrata* by Handel-Mazzetti who states that it approaches var. *intermedia* Muell. Arg.

Sapium rotundifolium Hemsley in Jour. Linn. Soc. Bot. XXVI. 445 (1894).—Handel-Mazzetti, Symb. Sin. VII. 212 (1931).

Baccaurea Esquirolii Léveillé, Fl. Kouy-Tchéou 159 (1914).—**Synon. nov.**

CHINA. Kweichou: Mou-you-se, *J. Cavalerie,* no. 2137 (no. 13), June 1904 "arbre, fl. jaunes" (syntype of *Baccaurea Esquirolii;* photo. in A. A.); Lo-fou, *J. Cavalerie,* no. 3458, Oct. 1908 (syntype of *B. Esquirolii;* photo. in A. A.); without precise locality, *J. Esquirol,* no. 517, June 1905 (syntype of *B. Esquirolii*; photo. in A. A.).

This species was collected in Kweichou also by Handel-Mazzetti (nos. 10279 and 10371).

DAPHNIPHYLLACEAE

Daphniphyllum macropodum Miquel in Mus. Bot. Lugd.-Bat. III. 129 (1867).—Rosenthal in Engler, Pflanzenr. IV.-147a, p. 9 (1919).

Webera Marchandii Léveillé in Fedde, Rep. Spec. Nov. XIII. 178 (1911); Fl. Kouy-Tchéou, 372 (1915).—**Synon. nov.**

CHINA. K w e i c h o u : moulins de Tong-tchéou, *J. Esquirol* & *R. Marchand,* no. 3252, June 22, 1912 (holotype of *Webera Marchandii;* photo. in A. A.).

BUXACEAE

Sarcococca Hookeriana Baill. var. **humilis** Rehder & Wilson in Sargent, Pl. Wilson. II. 164 (1914).

Maesa spec. Léveillé, Fl. Kouy-Tchéou, 287 (1914).

Myrsine Chevalieri Léveillé, Fl. Kouy-Tchéou, 287 (1914).— **Synon. nov.**

Pachysandra Mairei Léveillé, Cat. Pl. Yun-Nan, 97, fig. 23 (1916).

CHINA. K w e i c h o u : enfoncement de Ouan-ly près Thou-ly, *J. Esquirol,* no. 2593, Feb. 1911, "fleurs jaunâtres, fruits rouges" (holotype of *Myrsine Chevalieri;* merotype in A. A.). Y u n n a n : an pied de rochers, collines arides à l'est de Tong-tchouan, alt. 2600 m., *E. E. Maire,* March 1912 "arbrisseau toujours vert en touffes; fleurs blanches; fruits noirs" (holotype of *Pachysandra Mairei;* merotype in A. A.).

Esquirol's no. 2593, *Myrsine Chevalieri,* agrees exactly with specimens of *Sarcococca Hookeriana* var. *humilis* and I have no doubt that it belongs here, though Esquirol states that it has red fruits; the specimen before me bears only immature inflorescences. Under *Myrsine Chevalieri* Léveillé cites (l. c. p. 288) *Maesa* spec., Esquirol 2593, appearing on the preceding page as being the same. *Pachysandra Mairei* was identified by Handel-Mazzetti (Symb. Sin. VII. 235) with *S. Hookeriana* var. *digyna,* but I think it belongs with var. *humilis* which, however, may be only a dwarf and smaller form of var. *digyna.* A topotype or perhaps an isotype of *P. Mairei* was distributed as *Sarcocca* spec. E. E. Maire, no. 355, by the Arnold Arboretum.

Pachysandra stylosa Dunn in Jour. Bot. XLVI. 326 (1908).— Handel-Mazzetti, Symb. Sin. 236 (1931).

Pachysandra Bodinieri Léveillé in Fedde, Rep. Spec. Nov. XII. 187 (1913).

Pachysandra axillaris Franch. var. *Kouytchensis* Léveillé, Fl. Kouy-Tchéou, 166 (1914).

CHINA. K w e i c h o u : monts entre Ma-kay et Se-tchong-hien à Tien-sen-kiao, rochers à l'entrée du Tien-sen-kiao, *E. Bodinier,* no. 1525, Aug. 5, 1897 (holotype of *P. Bodinieri;* photo. in A. A.); Tsingay, *E. Bodinier* (holotype of *P. axillaris* var. *Kouytchensis;* photo. in A. A.).

Pachysandra Bodinieri is not enumerated in the Flore de Kouy-Tchéou, nor is Bodinier's no. 1525 cited under *Pachysandra.* According to Handel-Mazzetti both belong to *P. stylosa.*

Buxus microphylla S. & Z. var. **aemulans** Rehder & Wilson in Sargent, Pl. Wilson. II. 169 (1914).

Buxus Bodinieri in Fedde, Rep. Spec. Nov. XI. 549 (1913); Fl. Kouy-Tchéou, 160 (1914).—**Synon. nov.**

CHINA. K w e i c h o u : environs de Kouy-yang, mont du Collège, *E. Bodinier*, no. 2079, Feb. 25, 1898 "arbuste plus ou moins grand" (holotype of *B. Bodinieri;* merotype in A. A.).

The filaments in Bodinier's plant are longer than in the type of var. *aemulans*.

Buxus megistophylla Léveillé, Fl. Kouy-Tchéou, 160 (1914); Cat. Ill. Pl. Seu-Tchouen, pl. 26 (1918).

CHINA. K w e i c h o u : environs de Hoang-ko-chou, rocailles de la cascade, *J. Seguin* in herb. Bodinier, no. 2607, March 15, 1899 "arbuste de 0.60 cm., fleurs blanches" (syntype; merotype in A. A.); Kiao-men, près Lo-fou, *J. Esquirol,* no. 2560, Dec. 1910 (syntype; photo. in A. A.).

In its large leaves and subterete branchlets *B. megistophylla* resembles *B. Henryi* Mayr, but differs in the glabrous inflorescence, subsessile staminate flowers (in *B. Henryi* on pedicels 2-3 mm. long) and short stigmas; from *B. microphylla* S. & Z. it differs besides in the large leaves, chiefly in the short rudimentary ovary and the long filaments of the staminate flower.

Buxus Myrica Léveillé in Fedde, Rep. Spec. Nov. XI. 549 (1913); Fl. Kouy-Tchéou, 160 (1914).

Frutex ramulis tetragonis breviter pilosis gracilibus. Folia brevissime petiolata petiolis pilosulis, oblongo-lanceolata vel anguste lanceolata, 2-5 cm. longa et 0.5-1.4 cm. lata, acuta, mucronulata, basi cuneata, glabra, tenuiter coriacea et in sicco utrinque distincte reticulata, costa media utrinque elevata. Racemi axillares numerosi, rhachi dense pilosula elongata 5-7 mm. longa; bractearum paria 6-8, bracteae ovatae, acutae, 2 mm. longae, dorso dense pilosulae et intus ad marginem villosulis; flores ♂ breviter pedicellati pedicello 1-1.5 mm. longo dense pilosulo, sepalis ovalibus, 2 exterioribus carinatis carina ciliata, 2 interioribus longioribus glabris circ. 3 mm. longis, staminibus sepala superantibus 4 mm. longis, rudimento ovarii sepalis triplo breviore; flos ♀ terminalis, sepalis oblongo-ovatis 3-4 mm. longis, 3 exterioribus dorso dense 3 interioribus sparse pilosulis, ovario stylis complanatis apice recurvis multo breviore (ovarium 1.5 mm. longum, styli 3.5 cm. longi), stigmatibus ad medium stylum decurrentibus. Fructus non visus.

CHINA. K w e i c h o u : Pin-fa, *J. Cavalerie,* no. 3198, April 8, 1907 (syntype; merotype in A. A.); Lo-hou, *J. Esquirol,* no. 2566, Dec. 1910 (ex Léveillé; syntype); grande cascade de Hoang-ko-chou, dans

les rochers, *J. Seguin* in herb. Bodinier, no. 2266, April 3, 1898 (syntype; photo. in A. A.).

This species, of which I have given a description above, since that of Léveillé is inadequate, seems most closely related to *B. Henryi* Mayr, but differs in the quadrangular pilose branchlets, narrower and smaller thinly coriaceous and reticulate leaves, smaller and acute densely pilose bracts not scarious on the margin, in the shorter pedicels of the staminate flowers (in *B. Henryi* 2-3 mm. long) shorter filaments and shorter flattened styles. The two specimens which I have seen differ slightly in the size and shape of the leaves; Cavalerie's no. 3198 has the leaves mostly 3-5 cm. long and up to 1.4 cm. broad, while Bodinier's no. 2266 has comparatively narrower leaves 2-5 cm. long (a few even smaller) and 5-10 mm. broad; in the former the staminate flowers have mostly dropped while the latter is in full bloom.

Buxus Harlandi Hance var. **cephalantha** (Lévl. & Vant.), comb. nov.

Buxus cephalantha Léveillé & Vaniot in Fedde, Rep. Spec. Nov. III. 21 (1906).

Buxus sempervirens var. *microphylla* Léveillé, Fl. Kouy-Tchéou, 160 (1914).—Non Siebold & Zuccarini.

China. Kweichou: Pin-fa, rochers dans ou près ruisseaux, *J. Cavalerie*, no. 1797, Aug. 25, 1904 "tout petit buis, 1 pied de h." (holotype of *B. cephalantha;* photo. in A. A.).

This form differs from the type chiefly in its very small size; the plant is about 30 cm. high with short branchlets, the leaves measure 6-11 mm. in length and are mostly slightly emarginate and partly obovate (8:4 mm.), the inflorescences are mostly terminal.

CORIARIACEAE

Coriaria sinica Maximowicz in Mém. Acad. Sci. St. Pétersb. sér. 7, XXIX. no. III. 9, fig. (1881).—Léveillé Cat. Pl. Yun-Nan, 249 (1917).

Morus calva Léveillé in Fedde, Rep. Spec. Nov. XIII. 265 (1914).

China. Yunnan: coteaux arides à La-kou, alt. 2400 m., *E. E. Maire*, March 1912 "grand arbuste a l'écorce fibreuse, fleurs jaunâtres, d'abord rouges" (holotype of *Morus calva;* merotype in A. A.).

Morus calva was first identified with *Coriaria sinica* by C. Schneider (in Sargent, Pl. Wilson. III. 301. 1916).

ANACARDIACEAE

Spondias axillaris Roxburgh, Cat. Hort. Beng 34 (1814), nomen; Fl. Ind. II. 453 (1832).—Rehder & Wilson in Sargent, Pl. Wilson. II. 172 (1914).

Rhus Bodinieri Léveillé in Fedde, Rep. Spec. Nov. x. 437 (1912).—**Synon. nov.**

CHINA. Hongkong: bois de Happy Valley; bord de la routs à Pok-fu-lum, *E. Bodinier*, no. 1103, April 6 & 15, 1895 (holotype of *Rhus Bodinieri;* photo. in A. A.).

Pistacia chinensis Bunge in Mém. Div. Sav. Acad. Sci. St. Pétersb. II. 89 (Enum. Pl. Chin. Bor. 15) (1833).—Rehder & Wilson in Sargent, Pl. Wilson. II. 173 (1914).

Rhus gummifera Léveillé in Fedde, Rep. Spec. Nov. x. 474 (1912); Fl. Kouy-Tchéou, 412 (1915).—**Synon. nov.**

Rhus Argyi Léveillé in Mem. Acad. Ci. Barcelona, ser. 3, XII. 562 (Cat. Pl. Kiang-Sou, 22) (1916).—**Synon. nov.**

CHINA. Kiangsu: without precise locality, *Ch. d'Argy*, (1846-66) (holotype of *Rhus Argyi;* merotype in A. A.). Kweichou: au sud de Pin-fa près ruisseau, sur rochers, *J. Cavalerie*, no. 1078, June 18, 1903 "arbre produisant une espèce de gomme odorante" (holotype of *Rhus gummifera;* photo. and fragments in A. A.).

The specimen from Kiangsu has the rachis and the midrib of the leaflets below densely and finely pubescent while that from Kweichou is nearly glabrous. This in general seems to constitute a difference between the eastern and the western plants of this species; the specimens before me from the eastern provinces are mostly more or less pubescent on the rachis and the midrib of the leaflets, while the western specimens are glabrous or nearly so.

Rhus punjabensis Stewart var. **sinica** (Diels) Rehder & Wilson in Sargent, Pl. Wilson. II. 176 (1914).

? Rhus echinocarpa Léveillé in Fedde, Rep. Spec. Nov. x. 475 (1912); Fl. Kouy-Tchéou, 411 (1915), pro parte, quoad specim. Cavalerie, no. 2003.

Rhus Esquirolii Léveillé in Fedde, Rep. Spec. Nov. XII. 181 (1913); Fl. Kouy-Tchéou, 411 (1915).—**Synon. nov.**

? Rhus Mairei Léveillé, Sert. Yunnan. 2 (1916); Cat. Pl. Yun-Nan, 269 (1917).—**Synon. nov.**

CHINA. Kweichou: Tsin-gai, bois, *J. Cavalerie*, no. 1157, July 13, 1903, "petit arbre, fruit rouge-velour" (holotype of *Rh. Esquirolii;* photo. in A. A.). Yunnan: pied de montagne, derrière Tong-tchouan, alt. 2550 m., *E. E. Maire*, May 1912 "petit arbre cassant, rameaux rares, fleurs blanches" (syntype of *Rh. Mairei;* merotype in A. A.); haies de la plaine à Tong-tchouan, alt. 2500 m., July 1912 "fruit d'un petit arbre cassant, fl. blanches en grappes," *E. E. Maire* (syntype of *Rh. Mairei;* photo. in A. A.); plaine de Tche-hai, haies, alt. 2500 m., *E. E. Maire*, Aug. 1912 (ex Léveillé; syntype of *Rh. Mairei*).

Rhus Mairei I refer here with some doubt; it has partly only 5-7 leaflets and the rachis is not or very slightly winged, but otherwise it

seems to agree with *Rh. punjabensis* var. *sinica*. I also refer here doubtfully Cavalerie's no. 2003 numerated by Léveillé as a syntype of *Rh. echinocarpa*.

Rhus trichocarpa Miquel in Ann. Mus. Bot. Lugd.-Bat. II. 84 (1866); Prol. Fl. Jap. 16 (1866).—Rehder & Wilson in Sargent, Pl. Wilson. II. 180 (1914).

Rhus echinocarpa Léveillé in Fedde, Rep. Spec. Nov. x. 475 (1912); Fl. Kouy-Tchéou, 411 (1915); specim. no. 2003 excluso.—**Synon. nov.**

CHINA. K w e i c h o u : Mou-you-se, *J. Cavalerie*, no. 1016 (flowers), May 28, 1903 (syntype of *Rh. echinocarpa;* photo. and fragments in A. A.); without precise locality, fruit, *J. Cavalerie*, no. 108 (2) [?] (photo. in A. A.).

Under *Rhus echinocarpa* Léveillé cites two specimens, nos. 1016 and 2003, of which only the first belongs here, while the second is identical with *Rhus punjabensis* var. *sinica;* both are flowering specimens. The description of the fruit, from which the name of the species is derived, is apparently based on an unnamed specimen in the Léveillé herbarium marked only "J. Cavalerie, 108 (2)" on a slip and placed in the same cover with the other two specimens. As there is no other fruiting specimen of *Rhus* in the Léveillé herbarium which answers to the description of the very characteristic fruit of this species, this must be the specimen from which the description of the fruit was drawn, though it is not cited.

This record extends the range of *Rh. trichocarpa* west into Kweichou.

AQUIFOLIACEAE

Ilex suaveolens (Lévl.) Loesener in Ber. Deutsch. Bot. Ges. XXXII. 541 (1914).—Léveillé, Fl. Kouy-Tchéou, 201 (1914).

Celastrus suaveolens Léveillé in Fedde, Rep. Spec. Nov. XIII. 263 (1914).

CHINA. K w e i c h o u : environs de Kouy-yang, bois de Kien-lin-chan, *E. Bodinier*, no. 2663 [not 2683], June 19, 1899, "arbre de haute taille" (syntype of *Celastrus suaveolens;* photo. and fragments in A. A.); Pin-fa, *J. Cavalerie*, no. 17bis, June 3, 1902 "arbrisseau 5-6 m., fleurs blanches parfumées" (syntype of *C. suaveolens;* fragments in A. A.).

A full description of the species is given by Loesener (l. c.).

Ilex purpurea Hasskarl, Cat. Pl. Bogor. 230 (1844).—Loesener in Nov. Act. Leop.-Carol. Akad. LXXVIII. 111 (Monog. Aquifol.) (1901).

Callicarpa Cavaleriei Léveillé in Fedde, Rep. Spec. Nov. IX. 455 (1911); Fl. Kouy-Tchéou, 439 (1915).—**Synon. nov.**

Embelia rubro-violacea Léveillé in Fedde, Rep. Spec. Nov. x. 375 (1912); Fl. Kouy-Tchéou, 285 (1914).—**Synon. nov.**

Celastrus Bodinieri Léveillé in Fedde, Rep. Spec. Nov. XIII. 263 (1914).—**Synon. nov.**

CHINA. K w e i c h o u : environs de Tou-chan, *J. Cavalerie,* no. 2624, June 3, 1899 "grand arbuste, fleurs roses" (holotype of *Callicarpa Cavaleriei;* photo. in A. A.); Pin-fa, *J. Cavalerie,* no. 1334, June 23, 1902 (ex Léveillé; syntype of *Embelia rubro-violacea*); forêts, *J. Esquirol,* no. 429, June 1905, "grand arbrisseau" (syntype of *E. rubro-violacea;* merotype in A. A.); environs de Kouy-yang, mont du Collège, *E. Bodinier,* no. 2384, June 10, 1898 "arbuste" (holotype of *Celastrus Bodinieri;* photo. in A. A.).

The specimens cited above belong to var. *Oldhami* (Miq.) Loes. which represents the type of *I. purpurea* Hassk.; all are staminate plants in bloom.

Ilex pedunculosa Miq. var. **continentalis** Loesener in Nov. Act. Leop.-Carol. Akad. LXXVIII. 110 (Monog. Aquifol.) (1901); in Sargent, Pl. Wilson. I. 76 (1911).

Ilex purpurea var. δ *Leveilleana* Loesener in Léveillé, Fl. Kouy-Tchéou, 201 (1914).

CHINA. K w e i c h o u : Pin-fa, bois, *J. Cavalerie,* no. 1066, June 1903 (holotype of *I. purpurea* var. *Leveilleana;* photo. in A. A.).

Cavalerie's no. 1066 was considered by Loesener according to a note by him on the sheet of the type specimen, a possible hybrid between *I. purpurea* Hassk. and *I. pedunculosa* Miq., but the specimen seems to agree well in all respects with *I. pedunculosa* except that the petioles are not as deeply channelled as in *I. pedunculosa* and the margins of the leaves are somewhat more serrulate. In the solitary flowers (or in one case a 3-flowered cyme) and in the texture of the leaves it certainly agrees with *I. pedunculosa* which often has slightly serrulate leaves; the midrib above is short-pilose as it is, though usually to a lesser degree, in *I. pedunculosa;* in *I. purpurea* it is perfectly glabrous.

Ilex metabaptista Loes. var. **myrsinoides** (Lévl.), var. nov.

Maesa myrsinoides Léveillé in Fedde, Rep. Spec. Nov. X. 375 (1912); Fl. Kouy-Tchéou, 286 (1914).

Myrsine Feddei Léveillé in Fedde, Rep. Spec. Nov. X. 376 (1912); Fl. Kouy-Tchéou, 288 (1914).—**Synon. nov.**

Embelia Cavaleriei Léveillé, Fl. Kouy-Tchéou, 284 (1914).—**Synon. nov.**

Ilex Fargesii Franch. var. *Bodinieri,* Loesener apud Léveillé, l. c. 200 (1914).—**Synon. nov.**

Ilex metabaptista Léveillé, l. c. 200 (1914), vix Loesener.

CHINA. K w e i c h o u : Pin-fa, ruisseau de La-tong, *J. Cavalerie,* no. 579, Oct. 1, 1902 (holotype of *Maesa myrsinoides;* merotype in A. A.); Pin-fa, ruisseaux, *J. Cavalerie,* no. 842, May 23, 1902, "fleurs blanches" (holotype of *Myrsine Feddei;* merotype in A. A.); bords des

ruisseaux et torrents, *J. Cavalerie* in hb. Bodinier no. 2635, June 3, 1899, "fleurs blanches parfumées" (holotype of *Embelia Cavaleriei;* merotype in A. A.); environs de Kouy-yang, Mont du Collège, à la Cascade, *J. Chaffanjon,* May 28, 1898, et environs de Tou-chan, *J. Cavalerie,* June 3, 1899, in herb. Bodinier, no. 2310 (2 sheets, one is the holotype of *I. Fargesii* var. *Bodinieri,* the other represents *I. metabaptista* Lévl., vix Loes.; photos. in A. A.).

This variety differs from the type chiefly in the smaller leaves 2.5-4, rarely up to 6.5 cm. long, shorter 2-4 mm. long petioles and glabrous inflorescences. Of the three specimens cited above, Cavalerie, no. 579, is in fruit, while his no. 842 bears pistillate flowers and no. 2635 staminate flowers; the latter differs in the slightly broader elliptic-oblong leaves perfectly glabrous below, while the other two numbers are like the type sparingly short-pilose on the midrib below chiefly toward the base and pubescent on the midrib above. Bodinier's no. 2310 is represented by two sheets, one containing two staminate specimens, and named by Loesener *I. metabaptista,* while the other with one pistillate specimen is named by Loesener "*I. Fargesii* var. vel forma δ *Bodinieri* Loes. forma nova." The labels on each sheet contain the same information and give two localities, though one sheet has only a single specimen. All three are slightly different. The sheet named *I. metabaptista* by Loesener with two staminate specimens has slightly pilose inflorescences and thus approaching the type of the species, but the pubescence is less dense and the leaves are smaller than in the type. The one named *I. Fargesii* f. *Bodinieri* by Loesener is a pistillate specimen with narrow leaves up to 6.5 cm. long and glabrous inflorescences; on account of the latter character I refer it to var. *myricoides.* From *Ilex Fargesii* to which Loesener referred it, it is at once distinguished by the short petioles which are only half as long as the inflorescence, while in *I. Fargesii* the petioles are 1-1.5 cm. long and exceed the inflorescence, also the leaves are larger and broader and distinctly serrulate to below the middle.

The variety has also been collected in Kweichou by Y. Tsiang (no. 8525) near Tin-fan, Binshaw, and by Steward, Chiao and Cheo (no. 950) near Hung-shieh-lang, Kang-kou-hseh; in the latter specimen some of the leaves are up to 6 cm. long. Tsiang, no. 8525, was first identified by E. D. Merrill with *Maesa myrsinoides* Lévl. and distributed as *I. myrsinoides* (Lévl.) Merr.

Ilex corallina Franchet in Bull. Soc. Bot. France, XXXIII. 452 (1886).—Loesener in Nov. Act. Leop.-Carol. Akad. LXXVIII. 327 (Monog. Aquifol.) (1901).

Ilex Dunniana Léveillé in Fedde, Rep. Spec. Nov. IX. 458 (1911); Fl. Kouy-Tchéou, 200 (1914).—**Synon. nov.**

CHINA. Kweichou: Long-ly, *J. Cavalerie*, no. 3000, May, 1908, "petit arbre, fleurs jaunes" (holotype of *I. Dunniana;* merotype in A. A.).

Cavalerie's specimen represents a form with broad leaves rather closely denticulate with upright-spreading not incurved teeth. Léveillé had recognized the close relation of his species to *I. corallina*, but the differences he gives are either trivial or do not hold.

Ilex corallina Franch. var. **Loeseneri** Léveillé, Fl. Kouy-Tchéou, 200, (1914), nomen.

A typo recedit foliis angustioribus spinuloso-dentatis.

CHINA. Kweichou: Kouy-yang, mont du Collège, *J. Chaffanjon* in herb. Bodinier, no. 2242, Apr. 1898 (syntype; photo. in A. A.); Pin-fa, montagnes, *J. Cavalerie*, no. 580," arbrisseau, fl. odor." (syntype; photo. in A. A.).

The two specimens cited above bear Loesener's identification "*Ilex corallina* Franch. forma" on the sheets without any descriptive note, neither does Léveillé give a description. From the type this variety differs chiefly in the spinulose-serrate narrower leaves; in Cavalerie's no. 580 the spinulose mucro of the teeth is about 1 mm. long, but in Chaffonjon's specimens the mucro is much shorter and the leaves measure 1.5-2 cm. in width, while in Cavalerie's specimen they are 8-10 × 2-2.5 cm.

Ilex macrocarpa Oliver in Hooker, Icon. XVIII. t. 1787 (1888).—Loesener in Nov. Act. Leop.-Carol. Akad. LXXVIII. 489 (1901); Ber. Deutsch. Bot. Ges. XXXII. 543 (1914).—Léveillé, Fl. Kouy-Tchéou, 200 (1914).

Celastrus salicifolia Léveillé in Fedde Rep. Spec. Nov. XIII. 263 (1914).

Diospyros Bodinieri Léveillé, Fl. Kouy-Tchéou, 144 (1914).—**Synon. nov.**

CHINA. Kweichou: without precise locality, *J. Esquirol*, May 10, 1906 "arbre" (holotype of *Celastrus salicifolia;* photo. in A. A.); Kouy-yang, mont du Collège, *J. Chaffanjon* in herb. Bodinier, April 1898 "arbre" (holotype of *Diospyros Bodinieri;* merotype in A. A.).

Celastrus salicifolia was first identified with *Ilex macrocarpa* by Loesener (l. c.), and Léveillé enumerates in his Flore de Kouy-Tchéou the type of *C. salicifolia* under *I. macrocarpa* var. *genuina* Oliv., but does not cite *C. salicifolia* as a synonym.

CELASTRACEAE

Evonymus grandiflora Wallich in Roxburgh, Fl. Ind. ed. Carey. II. 404 (1824).—Loesener & Rehder in Sargent, Pl. Wilson. I. 484

(1913).—Loesener in Ber. Deutsch. Bot. Ges. XXXII. 540 (1914).—Léveillé, Cat. Pl. Yun-Nan, 34 (1915).

Evonymus Mairei Léveillé in Fedde, Rep. Spec. Nov. XIII. 260 (1914), excl. specim. "Siao-ho."

CHINA. Yunnan: brousse, pied de mont. à Tong-tchouan, alt. 2500 m., *E. E. Maire,* May 1912 "petit arbre, feuilles caduques, fleurs blanc-jaunâtre"; haies à pied de mont. à Tche-hai, alt. 2500 m., *E. E. Maire,* Aug. 1912 "petit arbre, toujours vert, fleurs jaunâtres, fruits item"; brousse des rochers â Ma-hong, alt. 2700 m., *E. E. Maire,* May 1912 "petit arbre, fleurs jaunes, fruits rouges"; (all three syntypes of *E. Mairei;* photos. in A. A.).

Evonymus Mairei was first identified with *E. grandiflora* by Loesener (l. c.).

Evonymus yunnanensis Franchet in Bull. Soc. Bot. France, XXXIII. 454 (1886).

Evonymus Mairei Léveillé in Fedde, Rep. Spec. Nov. XIII. 260 (1914), quoad specim. "Siao-ho."

CHINA. Kweichou: mont. derrière Siao-ho, alt. 2800 m., *E. E. Maire,* June 1912 "grande arbuste, feuilles caduques, fleurs jaunâtres" (syntype of *E. Mairei;* photo. in A. A.).

The specimen cited above was identified with *E. yunnanensis* by H. F. C[omber] according to a note on the sheet. Specimens recently collected in Kweichou by Y. Tsiang under nos. 9124 and 9133 belong here.

Evonymus uniflora Léveillé & Vaniot in Bull. Soc. Agr. Sci. Arts Sarthe, LIX. 320 (Bouquet Fl. Chine, 5) (1904); in Fedde, Rep. Spec. Nov. VI. 374 (1908); Fl. Kouy-Tchéou, 73 (1913).—Loesener in Ber. Deutsch. Bot. Ges. XXXII. 539 (1914).

Evonymus Blinii Léveillé in Fedde, Rep. Spec. Nov. XIII. 259 (1914), quoad specim. Esquirol, no. 478.

CHINA. Kweichou: Pin-fa, sud ouest, bord d'un ruisseau, *J. Cavalerie,* no. 256, Aug. 21, 1902 (holotype of *E. uniflora;* merotype in A. A.); without locality, *J. Esquirol,* no. 478, (fruit) (syntype of *E. Blinii,* and cited in Fl. Kouy-Tchéou; photo. in A. A.).

There are two entirely different specimens marked Esquirol, no. 478, in the Léveillé herbarium. One is a fruiting specimen, named on the label *E. uniflorus* by Léveillé; this is apparently the syntype of *E. Blinii,* upon which the description of the fruit was based. The other is a flowering specimen which bears the name *E. Blinii* in pencil in Léveillé's handwriting, which may be the specimen referred by Loesener to *E. theaefolia* Wall., but I am unable to distinguish this specimen which is in flower, from *E. Forbesiana* Loes.

This species has also been collected recently in Kweichou by Y. Tsiang (no. 4579).

Evonymus theaefolia Wallich, Cat. 4293 (1828), nomen.—M. A. Lawson in Hooker f., Fl. Brit. Ind. I. 612 (1875).—Léveillé, Fl. Kouy-Tchéou, 73 (1914).—Loesener in Ber. Deutsch. Bot. Ges. XXXII. 541 (1914).

? Evonymus Blinii Léveillé in Fedde, Rep. Spec. Nov. XIII. 259 (1914) quoad specim. "Esquirol, no. 478."

The syntype of *E. Blinii,* J. Esquirol, no. 478 (flowers), was first identified by Loesener (l. c.) with *E. theaefolia* Wall. as "*E. theifolia* var. vel forma" and is cited by Léveillé as a synonym of the latter. The specimen, however, of *E. Blinii,* Esquirol, no. 478, which I have before me, I cannot distinguish from *E. Forbesiana* Loes., but there may be or may have been another specimen of the same number which I have not seen and which may belong to *E. theaefolia* Wall. See also my remarks under the preceding species.

Evonymus theaefolia Wall. occurs in Kweichou, as Y. Tsiang's no. 7274 shows which was collected in 1930 near Gan-wu, Lohou in southern Kweichou.

Evonymus Esquirolii Léveillé in Fedde, Rep. Spec. Nov. XIII. 261 (1914); Fl. Kouy-Tchéou, 71 (1914).—Loesener in Ber. Deutsch. Bot. Ges. XXXII. 539 (1914).

CHINA. K w e i c h o u : Tang-tchang (Hoang-tsao-pa), *J. Esquirol,* no. 1569, June 1909 (holotype; merotype in A. A.).

Related to *E. myriantha* Hemsl. according to Loesener from which it differs chiefly in the much smaller leaves and denser less branched cymes.

Evonymus Leclerei Léveillé in Fedde, Rep. Spec. Nov. XIII. 260 (1914); Fl. Kouy-Tchéou, 72 (1914).—Loesener in Ber. Deutsch. Bot. Ges. XXXII. 539 (1914).

CHINA. K w e i c h o u : Ma-jo, *J. Cavalerie,* no. 3058, Sept. 5, 1907 (holotype; merotype in A. A.).

This species is like the preceding closely related to *E. myriantha* Hemsl. from which it differs in the longer leaves more densely and distinctly reticulate above.

Evonymus centidens Léveillé in Fedde, Rep. Spec. Nov. XIII. 262 (1914); Cat. Pl. Yun-Nan, 34 (1915).—Loesener, in Ber. Deutsch. Bot. Ges. XXXII. 262 (1914).—Rehder in Jour. Arnold Arb. XI. 164 (1930).

CHINA. Y u n n a n : collines broussailleuses a Long-ky, alt. 700 m., *E. E. Maire,* June 1912 "grand arbuste à feuilles caduques, fleurs jaunâtres, fruits rouges" (holotype; merotype in A. A.).

Of this species I gave a full description in 1930 (l. c.) based on the specimen cited above and Fang's no. 5819.

Evonymus Dielsiana Loes. var. γ **latifolia** Loesener in Bot. Jahrb. xxx. 455 (1902); in Ber. Deutsch. Bot. Ges. xxxii. 540 (1914).

Evonymus Cavaleriei Léveillé in Fedde, Rep. Spec. Nov. xiii. 259 (1914).

Evonymus Dielsiana Léveillé, Fl. Kouy-Tchéou, 71 (1914), non Loesener sensu stricto.

China. Kweichou: Pin-fa, rochers de Ouen-pi, Li-tseou-gai, *J. Cavalerie,* no. 87, July 23, 1902 "haut 3-4 m." (syntype of *E. Cavaleriei;* merotype in A. A.); Pin-fa, *J. Cavalerie,* no. 865, Feb. 17, 1903 (syntype of *E. Cavaleriei;* photo. in A. A.).

Evonymus Cavaleriei Léveillé in Fedde (not in his Fl. Kouy-Tchéou) was first identified with *E. Dielsiana* var. *latifolia* by Loesener, but Léveillé in his Flore de Kouy-Tchéou omits the variety.

Evonymus Feddei Léveillé in Fedde, Rep. Spec. Nov. xiii. 260 (1914); Fl. Kouy-Tchéou, 72 (1914).—Loesener in Ber. Deutsch. Bot. Ges. xxxii. 539 (1914).

China. Kweichou: route de Pin-fa à Lo-fou, *J. Cavalerie,* no. 3353, April 1908 (holotype; merotype in A. A.).

According to Loesener this species is related to *E. attenuata* Wall. and *E. bullata* Wall.; from the former it differs in the much larger leaves and more branched inflorescences and from the latter in the narrower leaves and less branched inflorescences.

Evonymus Rehderiana Loesener in Sargent, Pl. Wilson. i. 488 (1913); in Ber. Deutsch. Bot. Ges. xxxii. 540 (1914).—Léveillé Fl. Kouy-Tchéou, 73 (1914).

Evonymus bicolor Léveillé in Fedde, Rep. Spec. Nov. xiii. 260 (1914).

Evonymus proteus Léveillé, l. c.

China. Kweichou: Long-ly, Ma-jo, *J. Cavalerie,* no. 2238 (in part), Nov. 13, 1907 "arbre" (holotype of *E. bicolor;* photo. in A. A.); Paitchen, *J. Cavalerie,* no. 2238 (in part), March 30, 1905 "3 or 4 m. de haut, les fleurs et les jeunes feuilles vert-jaunes" (holotype of *E. proteus;* photo. in A. A.).

Evonymus bicolor and *E. proteus* were first identified with *E. Rehderiana* by Loesener (l. c. 1914). Both specimens cited bear the number 2238. The published name *E. proteus* for the specimen from Paitchen does not appear on the original label, but instead another name referring to the color of the branches. Also collected in Kweichou by Y. Tsiang (nos. 5121 and 9029).

Evonymus Forbesiana Loesener in Bot. Jahrb. XXX. 457 (1902); in Ber. Deutsch. Bot. Ges. XXXII. 540 (1914).—Léveillé, Fl. Kouy-Tchéou, 72 (1914).

Evonymus Crosnieri Léveillé in Bull. Soc. Bot. France, LI. p. cxlvi (1904).

Evonymus Blinii Léveillé in Fedde, Rep. Spec. Nov. XIII. 250 (1914), quoad specim. "Esquirol, no. 478."

Evonymus Vanioti Léveillé, l. c. (1914).

CHINA. Kweichou: Pin-fa, bord des ruisseaux, *J. Cavalerie*, no. 1274, June 2, 1902 "fl. rousse" (holotype of *E. Crosnieri;* photo. in A. A.); Pin-fa, *J. Cavalerie*, no. 1272, March 10, 1902 "fl. rousse" (syntype of *E. Vanioti;* photo. in A. A.); moulins de Tong-tchéou, *J. Esquirol*, no. 3236, July 10, 1912 (syntype of *E. Vanioti;* photo. in A. A.); Pin-fa, *J. Cavalerie*, no. 1273, May 5, 1902 (syntype of *E. Blinii;* photo. in A. A.); without precise locality, "coteaux," *J. Esquirol*, no. 478 (flowers), June 1905 (? syntype of *E. Blinii;* photo. in A. A.).

Evonymus Crosnieri, E. Vanioti and one of the syntypes of *E. Blinii* were first referred to *E. Forbesiana* by Loesener (l. c.), and the first two names placed as synonyms under *E. Forbesiana* by Léveillé in his Flore de Kouy-Tchéou. *Evonymus Blinii* is not cited as a synonym, but Esquirol's no. 478, one of the syntypes of that species which, however, partly belongs to *E. uniflora* Lévl., while Cavalerie, no. 1273, which belongs here, is not cited.

Evonymus aculeata Hemsley in Kew Bull. Misc. Inform. 1893, p. 209.—Sprague in Kew Bull. Misc. Inform. 1908, p. 33.

Echinocarpus hederaerhiza Léveillé in Fedde, Rep. Spec. Nov. X. 474 (1912).—**Synon. nov.**

Evonymus acanthocarpa Léveillé, Fl. Kouy-Tchéou, 71 (1914); non Franchet.

CHINA. Kweichou: Pin-fa, rampant sur les rochers, *J. Cavalerie*, no. 2761, April 1906 (holotype of *Echinocarpus hederaerhiza;* photo. in A. A.).

Echinocarpus hederaerhiza was referred by Léveillé in his Flore de Kouy-Tchéou to *Evonymus acanthocarpa* Franch., but it differs from that species chiefly in the longer compressed spines of the fruit, in the black bud-scales persisting at the base of the branchlets for some time and in the non-verruculose branchlets.

Evonymus acanthocarpa Franchet, Pl. Delavay. 129 (1889).—Sprague in Kew Bull. Misc. Inform. 1908, p. 32.

Echinocarpus erythrocarpa Léveillé in Fedde, Rep. Spec. Nov. X. 474 (1912).—**Synon. nov.**

Evonymus erythrocarpa Léveillé, Fl. Kouy-Tchéou, 72 (1914).

CHINA. Kweichou: environs de Gan-pin, dans la montagne,

L. Martin in hb. Bodinier, no. 2493, Oct. 9, 1898 "arbuste lianeux" (holotype of *Echinocarpus erythrocarpa;* merotype in A. A.).

Evonymus subtrinervis, nom. nov.

Echinocarpus Esquirolii Léveillé in Fedde, Rep. Spec. Nov. x. 474 (1912); non *Evonymus Esquirolii* Léveillé.—**Synon. nov.**

Echinocarpus Cavalerici Léveillé, l. c.—**Synon. nov.**

Evonymus Blinii Léveillé, Fl. Kouy-Tchéou, 71 (1914); non Léveillé in Fedde, Rep. Spec. Nov. XIII. 259 (1914).

Evonymus Cavalerici Léveillé, Fl. Kouy-Tchéou, 71 (1914); non Léveillé in Fedde, Rep. Spec. Nov. XIII. 259 (1914).

Frutex glaber, ramis gracilibus tenuiter verruculosis quadrangulatis angulis subalatis; gemmae terminales parvae pauciperulatae, obtusae, pallide cinereo-virescentes. Folia persistentia chartacea, ovato-oblonga vel oblongo-lanceolata, 4.5-9 cm. longa et 1.8-3 cm. lata, acuminata, basi late cuneata vel rarius fere rotundata, fere ad basin denticulata denticulis mucrone fusco inflexo, basi trinervia, costa medio utrinque elevata, nervis lateralibus 4-6, pari inferiore superioribus aequilongo vel longiore, supra in sicco manifeste, subtus leviter elevatis, supra laete viridia, reti nervulorum in sicco visibili, subtus pallida, reti obsoleto; petioli 2-4 mm. longi, tenues. Flores desiderantur. Inflorescentiae fructiferae bis dichotomae; pedunculus circiter 1.5 cm. longus, gracilis, quadrangularis, ramulis circiter 1 cm., pedicellis 2-3 mm. longis: capsula subglobosa, aculeis inclusis circiter 1 cm. diam., pallide flavo-carnea, aculeis complanatis 1-1.5 mm. longis, dissitis non dense aggregatis; semina 6 mm. longa, nigra, arillo pallide aurantiaco apice laciniato aperto excepto inclusa.

CHINA. K w e i c h o u : without precise locality, *J. Esquirol,* no. 844 (holotype of *Echinocarpus Esquirolii, E. Blinii* and *E. subtrinervis;* photo. and merotype in A. A.); without precise locality, *J. Esquirol* (holotype of *Echinocarpus Cavaleriei;* photo. in A. A.).

The specimens cited above I have not been able to identify with any previously described species; they seem nearest to *E. echinata* Wall. from which they are easily distinguished by the leaves being 3-nerved at the base, with fewer veins diverging at a more acute angle. From *E. Hemsleyana* Loes. they differ in the shape of the leaves, shorter petioles, and the shorter spines of the fruit and smaller winter-buds. As Léveillé's description is insufficient I have drawn up a more complete description based on Esquirol no. 844. The other specimen, the type of *Echinocarpus Cavaleriei,* is rather poor with all the leaves dropped from the branches and very young fruit; it differs from the type in the broader leaves, ovate-oblong, partly rounded at base, about 5 cm. long and 2.2 cm. broad.

Unfortunately none of the names proposed by Léveillé can be used

for this species. The two specific epithets under *Echinocarpus* cannot be transferred to *Evonymus* since the resulting combinations are preoccupied and *Evonymus Blinii*, based on *Echinocarpus Esquirolii* is invalidated by an earlier homonym, which by Léveillé was considered non-valid, because it had been reduced to synonymy. It should be noted here that in 1914 three important publications relating to *Evonymus* came out: Léveillé's descriptions of many new species in Fedde's Repertorium on May 5, 1914, Loesener's corrections in Ber. Deutsch. Bot. Ges. on July 30, 1914, and Léveillé's Flore de Kouy-Tchéou which contains these corrections, in September or October of that year.

Evonymus Maackii Ruprecht in Bull. Phys. Math. Acad. Sci. St. Pétersb. XV. 358 (1857).—Loesener in Ber. Deutsch. Bot. Ges. XXX. 540 (1914).—Léveillé, Fl. Kouy-Tchéou, 71 (1914).

Evonymus coreanus Léveillé in Fedde, Rep. Spec. Nov. VIII. 284 (1910).

KOREA: in dumosis Chinnampo, *U. Faurie*, no. 520, Aug. 1906 (holotype of *E. coreanus*, isotype in A. A.).

Evonymus coreanus was first referred to *E. Maackii* by Loesener (l. c.).

Evonymus Hamiltoniana Wallich in Roxburgh, Fl. Ind. ed. Carey, II. 403 (1824).—Loesener in Ber. Deutsch. Bot. Ges. XXX. 541 (1914).—Léveillé, Fl. Kouy-Tchéou, 72 (1914).

Evonymus rugosa Léveillé in Fedde, Rep. Spec. Nov. XIII. 261 (1914).

Evonymus Darrisii Léveillé, l. c.—**Synon. nov.**

Evonymus Maackii Léveillé, Fl. Kouy-Tchéou 73 (1914); non Ruprecht.

CHINA. Kweichou: Hoang-tsao-po, colline de la pagode, *J. Esquirol*, no. 1532 (holotype of *E. rugosa;* photo. in A. A.); without precise locality, *J. Esquirol*, no. 711 (holotype of *E. Darrisii;* photo. in A. A.).

Evonymus rugosa was first referred to *E. Hamiltoniana* by Loesener with some doubt, while he identified *E. Darrisii* with *E. Maackii*. Though *E. Darrisii* in its leaves resembles *E. Maackii* rather than *E. Hamiltoniana*, the fruit seems to agree better with that of the latter species. Also for geographical reasons it seems unlikely that the Kweichou plant belongs to *E. Maackii* which has not yet been recorded from central and western China.

Evonymus lanceifolia Loesener in Bot. Jahrb. XXX. 462 (1902); in Ber. Deutsch. Bot. Ges. XXXII. 541 (1914).—Léveillé, Fl. Kouy-Tchéou, 72 (1914).

Evonymus Bodinieri Léveillé in Fedde, Rep. Spec. Nov. XIII. 261 (1914).

China. Kweichou: Gan-chouen, *J. Cavalerie*, no. 3824, June 1910 "petit arbre" (holotype of *E. Bodinieri;* photo. in A. A.).

Evonymus Bodinieri was referred to *E. lanceifolia* by Loesener with the remark that it approaches *E. Hamiltoniana*. The type specimen consists of two branches, a flowering and a fruiting branch; according to the dark colored anthers the specimen belongs to *E. lanceifolia*, for *E. Hamiltoniana* differs from both closely related species *E. Maackii* and *E. lanceifolia* in the yellowish white anthers. The label of the type specimen bears in Léveillé's handwriting an unpublished name referring to the color of the leaves, to which is added rather indistinctly written in pencil "vel Bodinieri."

Evonymus alata (Thbg.) Regel, Fl. Ussur. 40, t. 7 (1861).—Loesener & Rehder in Sargent, Pl. Wilson. I. 493 (1913).

Microrhamnus Taquetii Léveillé in Fedde, Rep. Spec. Nov. VIII. 284 (1910).—**Synon. nov.**

Korea. Quelpaert: secus torrentes, *T. Taquet*, no. 153, Sept. 1907 (holotype of *Microrhamnus Taquetii;* fragments of type and isotype in A. A.).

Besides the type specimen the cover of *Microrhamnus Taquetii* contains Taquet, no. 4095, consisting of flowering branches of *E. alata;* both specimens belong to the form *aptera* Regel with the branches not or very slightly winged.

Evonymus disticha Léveillé in Fedde, Rep. Spec. Nov. XIII. 261 (1914); Fl. Kouy-Tchéou, 71 (1914).—Loesener in Ber. Deutsch. Bot. Ges. XXXII. 540 (1914).

China. Kweichou: environs de Kouy-yang, bois de la pagode de Kien-lin-chan, *E. Bodinier*, no. 2455, June 27, 1898 "petit arbuste, fleurs jaunes" (holotype, merotype in A. A.).

This species was collected also at the same locality in 1930 by Y. Tsiang, no. 8488. This specimen bears flowers and young fruits which differ from those of *E. alata* in their shorter and broader lobes.

Celastrus Vanioti (Lévl.), comb. nov.

Saurauja Vanioti Léveillé, Fl. Kouy-Tchéou, 415 (1915, before October.)

Celastrus spiciformis Rehder & Wilson in Sargent, Pl. Wilson. II. 348 (1915, Dec. 28).—**Synon. nov.**

China. Kweichou: environs de Kouy-yang, route du Collège, dans les haies, *E. Bodinier*, May 30, 1898 "liane ligneuse" (holotype of *Saurauja Vanioti;* photo. and merotype in A. A.).

Saurauja Vanioti is without doubt identical with *Celastrus spiciformis*, the former differing only slightly in the somewhat more reticulate leaves resembling in this respect *C. gemmata* Loes., but paler on the under

surfaces. As *Saurauja Vanioti* was published several months earlier, the Flore de Kouy-Tchéou having been received at the Arnold Arboretum in October 1915, the transfer of the specific epithet to *Celastrus* becomes necessary.

Celastrus gemmata Loesener in Bot. Jahrb. XXX. 468 (1902).—Rehder & Wilson in Sargent, Pl. Wilson. II. 352, (1915).

Embelia Esquirolii Léveillé in Fedde, Rep. Spec. Nov. X. 374 (1912).—**Synon. nov.**

CHINA. Kweichou: Collège, *J. Esquirol,* no. 4, May 10, 1906 "arbre, fleurs blanc-verdâtres" (holotype of *Embelia Esquirolii,* merotype in A. A.).

Celastrus gemmata has been collected in Kweichou by Y. Tsiang under nos. 4661, 5360 and 6450.

Celastrus stylosa Wallich in Roxburgh, Fl. Ind. ed. Carey, II. 401 (1924).—Léveillé, Fl. Kouy-Tchéou, 69 (1914).

Celastrus Cavalerici Léveillé in Monde Pl. sér. 2, XVIII. 31 (1916); non Léveillé (1914).

CHINA. Kweichou: Pin-fa, ruisseaux, *J. Cavalerie,* no. 496, Sept. 16, 1902 (holotype of *C. Cavaleriei;* photo. in A. A.).

Celastrus Cavaleriei was by Loesener according to his note on the type sheet referred to *C. stylosa* or a related species.

Celastrus flagellaris Ruprecht in Bull. Acad. Sci. St. Pétersb. sér. 3, XV. 357 (1857); Decas Pl. Amur. t. 4 (1859).—Loesener in Ber. Deutsch. Bot. Ges. XXX. 541 (1914).—Rehder & Wilson in Sargent, Pl. Wilson. II. 357 (1915).

Celastrus clemacanthus Léveillé in Fedde, Rep. Spec. Nov. VIII. 284 (1910).

KOREA. Quelpaert: scandens in muris agrorum Haouen, *T. Taquet,* no. 632, May 8, 1908 (holotype of *C. clemacanthus;* photo. and isotype in A. A.).

Celastrus clemacanthus Léveillé was identified with *C. flagellaris* by Loesener (l. c.).

Celastrus Hindsii Benth. var. **Henryi** Loesener in Bot. Jahrb. XXIX. 444 (1900); XXX. 467 (1902).

Erythrospermum Cavalerici Léveillé, Fl. Kouy-Tchéou, 51 (1914).—**Synon. nov.**

CHINA. Kweichou: Gan-chouen, rochers, *J. Cavalerie,* no. 3976, in 1912, "liane" (holotype of *Erythrospermum Cavaleriei;* merotype in A. A.).

Gymnosporia Esquirolii Léveillé in China Rev. Ann. 1916, p. 18 (1916).—Schneider in Oester. Bot. Zeitschr. LXVII. 140, in nota (1918).

Berberis Cavalerici Léveillé, Fl. Kouy-Tchéou, 48 (1914); non Léveillé in Fedde, Rep. Spec. Nov. IX. 454 (1911).

Berberis Esquirolii Léveillé, Fl. Kouy-Tchéou, 47, in clavi (1914).

China. Kweichou: Lo-fou, colline du fort, 800 m., terrain rocheux aride, *J. Esquirol,* no. 3645, June 20, 1912 (holotype of *Berberis Cavaleriei;* merotype in A. A.).

Gymnosporia Esquirolii is closely related to *G. diversifolia* Maxim. but easily distinguished by the slender peduncles of the inflorescence 0.5-1.5 cm. long and by the closely and finely crenulate or serrulate leaves, often acute at the apex and of thinner texture. To this species I also refer Siméon Ten, nos. 169 and 378, from Pe-yen-tsin, Henry, no. 9391, from Mengtze, and Forrest no. 10738. *Gymnosporia Esquirolii* seems to be restricted to Yunnan and Kweichou, while *G. diversifolia* Maxim. is widely distributed through southeastern China, Indochina, Formosa and the Liukiu Islands.

Gymnosporia Esquirolii was published without reference to a specimen, only with the citation of *Berberis Cavaleriei* as a synonym. This citation refers without doubt to *B. Cavaleriei* Léveillé, Fl. Kouy-Tchéou, 48, which appears in the key on the preceding page as *B. Esquirolii.* This species is based on Esquirol's no. 3645 and fragments were sent to C. Schneider by Léveillé in November 1915 as *B. Esquirolii,* but without indication of collector and number. These fragments agree with the specimen cited above and were referred by Schneider to *Gymnosporia* (in Sargent, Pl. Wilson. II. 359). Léveillé apparently had first given to Esquirol's no. 3645 the name *B. Esquirolii,* but when Schneider, to whom Léveillé had sent in October 1913 specimens of his earlier *B. Cavaleriei* based on Cavalerie, no. 3209, and published in 1911 (in Fedde Rep. Spec. Nov. IX. 454), had referred this species to *B. Griffithiana* Wall., he changed the name of his not yet published *B. Esquirolii* to *B. Cavaleriei,* wishing to keep Cavalerie's name attached to a valid species, but forgot to change the name in the key. *Berberis Cavaleriei* of 1911, however, which was in 1913 considered a synonym of *B. Griffithiana* by Schneider, and was accepted as such by Léveillé in his Flore de Kouy-Tchéou with the citation of "*B. Cavaleriei* Lévl. olim" as a synonym, was revived as a valid species by Schneider in 1918 (in Oesterr. Bot. Zeitschr. LXVII. 140) and applied to the Chinese species formerly referred to *B. Griffithiana* which according to his revised opinion does not occur at all in China.

Gymnosporia acuminata Hooker f., Fl. Brit. Ind. I. 619 (1875).

Evonymus yunnanensis Léveillé, Fl. Kouy-Tchéou, 73 (1914); non Franchet.

China. Kweichou: Lo-fou, *J. Cavalerie,* no. 3530, March 1909 (cited under *E. yunnanensis* Franch. by Léveillé; photo. in A. A.).

The specimen cited above differs from typical *G. acuminata* of which

I have isotypes of Hooker's and Griffith's specimens before me, in the smaller and narrower scarcely acuminate leaves 7-9 cm. long and 2-3 cm. broad and rather closely serrulate to near the base, but fruit and inflorescence does not seem to differ. It looks rather different and I was inclined to consider it at least a distinct variety, if not Hemsley's no. 13437 from Szemao represented an intermediate form, agreeing in the size of the leaves with the type and in the serration, though less dense and fine, with Cavalerie's specimen.

Tripterygium hypoglaucum (Lévl.) Hutchinson in Kew Bull. Misc. Inform. 1917, p. 101.—Léveillé, China Rev. Ann. 1916, p. 23 (Mscr.).

Aspidopterys hypoglauca Léveillé in Fedde, Rep. Spec. Nov. IX. 458 (1911).

Pentace Virginis Léveillé in Monde Pl. sér. 2, XVIII. 28 (1916).—**Synon. nov.**

CHINA. K w e i c h o u : Ma-jo, *J. Cavalerie,* no. 3316, Aug. 1908 "petit arbre lianeux" (holotype of *Aspidopterys hypoglauca;* merotype in A. A.). Y u n n a n : brousse du plateau de Ie-ma-tchouan, 3300 m., *E. E. Maire,* June 1912 "arbuste grimpant, long rameaux, fleurs blanches, fruit rouge sombre" (holotype of *Pentace Virginis;* photo. in A. A.).

Hutchinson first recognized *Aspidopterys hypoglauca* as a *Tripterygium* and communicated the resulting new combination to Léveillé who published it in his "China; revue annuelle 1916" distributed as manuscript to the larger botanical institutions before Hutchinson's paper was printed.

(*To be continued*)

HERBARIUM, ARNOLD ARBORETUM,
HARVARD UNIVERSITY.

VARIATION IN FLOWER COLOR IN HAMAMELIS VERNALIS

EDGAR ANDERSON

With one text figure

WHATEVER the ultimate explanation, there seem to be certain species of plants in which the variation between one individual and another is unusually marked. *Hamamelis vernalis* is such a species, for the plants of a single locality vary markedly in flower color, color pattern, time of blooming, and the number and arrangement of the flowers on the flowering branches. In this respect the species stands in sharp contrast to *H. virginiana* which, though it runs into geographical varieties, is comparatively uniform in any one locality. In *Hamamelis virginiana*, variation in flower color, while not unknown, is extremely rare. Examination of many individuals in eastern Massachusetts has revealed only two with a faint flush of red at the base of the petals. At Sterling, Massachusetts, for instance, of 35 individuals examined, 34 were pure yellow and one was flushed with red on the calyx and at the base of the petals. There is at the Arnold Arboretum a bush presumably collected in eastern Massachusetts which has light red petals and a yellowish green calyx (Rehder, 1922). A similar bush was collected some years ago near Malden, Massachusetts, by Mr. Edward L. Rand (Sargent, 1893). Mr. O. A. Farwell has collected a similar form with red calyx lobes at Stony Creek, Michigan and it has been distributed as Farwell, no. 3943. Variation in flower color is therefore not unknown in *H. virginiana*, though it is comparatively infrequent.

In *H. vernalis* nearly every bush has its own distinctive flower color and it is not at all uncommon to find pure yellow-flowered plants growing side by side with red-flowered ones. These differences seem to be germinal, since there is little variation between the flowers on a single bush and since the peculiarities of a particular bush persist after transplantation. The specimens in the living collections of the Arnold Arboretum maintain their characteristic flower colors season after season and the colors are perpetuated in plants propagated vegetatively. One color form has already received taxonomic recognition, *Hamamelis vernalis* forma *carnea* Rehd.

The following records of variation in natural populations of *H. vernalis* (Tables 1 & 2) were made in St. Francis County, Missouri, on February 28, 1931. In each case the bushes, as is usual in this species, were growing in gravelly creek beds. At each locality, for a distance of

approximately one-eighth of a mile along the watercourse, a single representative twig was taken from each bush then in flower. The specimens were then taken to the laboratory and a record was made of their color and color pattern. The underlying yellow pigment seems to be practically the same shade throughout all the plants and nearly all of the variation in color is due to variations in the intensity and distribution of the red pigment, which was found to be a water soluble substance occurring in the epidermis of the petals and sepals. In the petals it varies from none at all, to a faint flush at the base, through intermediate stages up to 85% of the length. In their general tone the flowers varied from Light Cadmium Yellow (Ridgway) to Dragon's Blood Red (Ridgway). The coloring always extends up from the base of the petals and even the reddest flowers were yellow at the very tips of the petals. On the sepals the color seems to spread from the mid-vein. In the following records it has been summarized under four grades:

"pure yellow."	no red pigment in the sepal.
"line plus."	sepal red along the mid-vein with a faint flush of red at either side.
"all but margin."	sepal red with a narrow yellow margin at either side.
"entire."	red pigment distributed over the entire sepal.

It will be seen that while the variation at the two localities was similar, that the colors were on the average a little darker at Flat River than at Libertyville. While the intensity of the red coloring on the petals was associated with the coloring of the sepal, the correlation was not perfect. This is shown graphically in Table 2 which summarizes the data from both localities.

The above tables present a graphic and objective summary of variation in color pattern. The size and development of the flowering branches seem to be quite as variable, though it is difficult to record the variation objectively. My colleague, Mr. Ernest J. Palmer, tells me that there is a correspondingly great variation in the degree of pubescence of the leaves. This has been given varietal recognition in *H. vernalis* var. *tomentella* (Rehd.) E. J. Palmer.

It is particularly interesting to find such variation in *H. vernalis* since it is a species of very limited distribution, being known only from the Ozark Mountains and adjacent lowlands to the south and west. It may well be an ancient species like many others in that area (Palmer and Steyermark, in press), since its closest affinities are with the Asiatic species of *Hamamelis* rather than with the American *H. virginiana*. Like them it is winter-flowering, like them it has an extensive develop-

ment of red pigment in the flower, like some of them it has a tendency to pubescent leaves. The genus is known from its fossil record (Berry, 1916) to be an ancient one which was formerly wide-spread in the northern hemisphere.

It is commonly said that species of limited distribution are less variable than those more widely distributed. As far as *Hamamelis vernalis* and *H. virginiana* are concerned the reverse seems to be true. The approximate ranges of the two species are shown in Figure 1. *Hamamelis virginiana* covers an area many times as large as that of *H. vernalis* and yet in any one locality the variation in color, habit, and pubescence is much less. It is of course quite possible that variability

TABLE 1. Variation in petal color and sepal color of 63 plants of *Hamamelis vernalis* examined at Libertyville and Flat River, Missouri. Further explanation in the text.

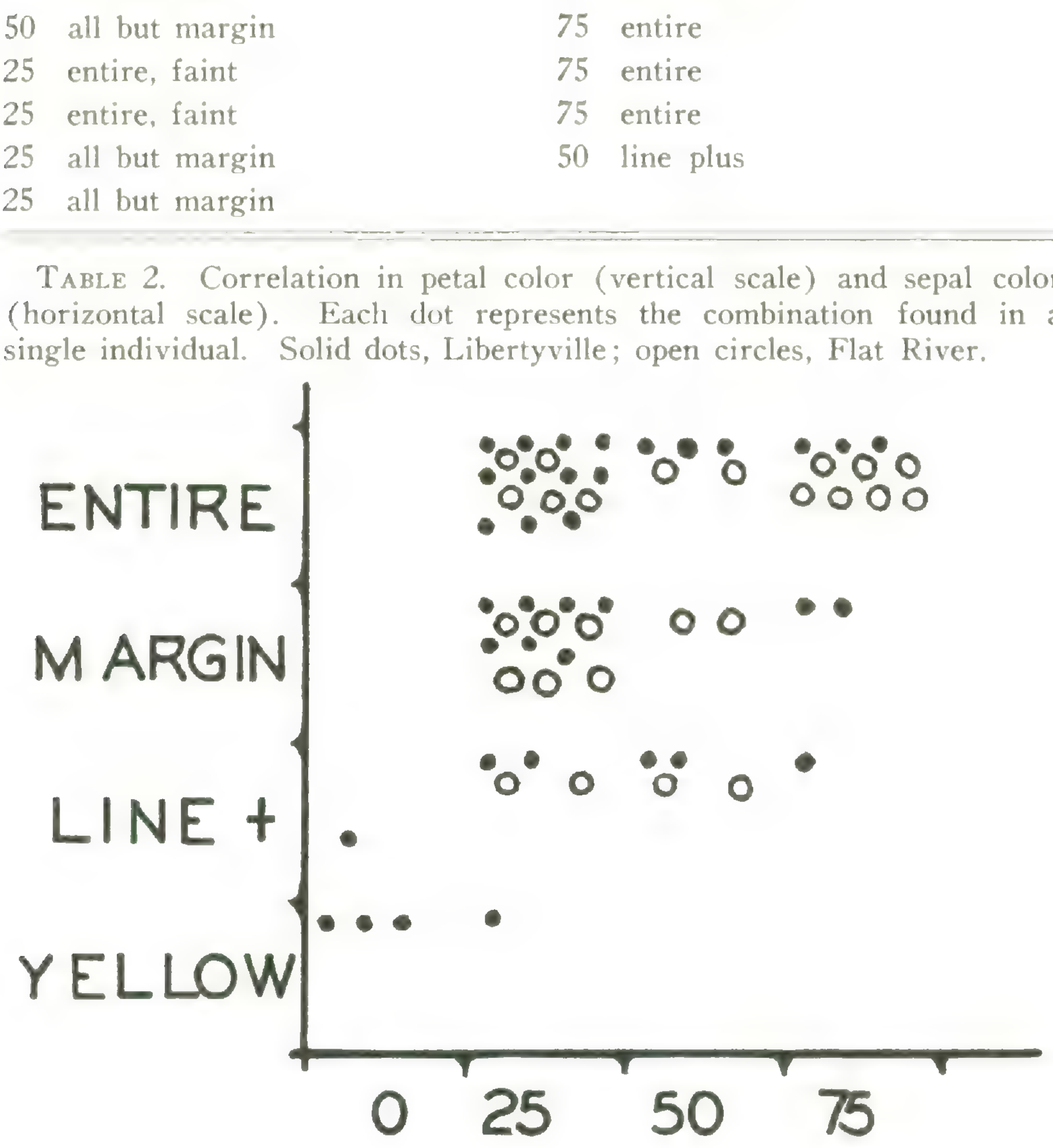
Libertyville, Missouri

% of petal red	distribution of red in calyx	% of petal red	distribution of red in calyx
0	pure yellow, flush of red at petal base	50	line plus
25	line plus	50	entire, faint
75	entire	25	entire, faint
25	entire, faint	25	entire
50	entire, faint	25	entire
25	all but margin	75	all but margin
0	line plus	25	entire, faint
25	entire, faint	25	all but margin
50	entire, dark	25	entire
25	entire, faint	25	pure yellow
25	entire	25	entire
0	pure yellow, pure lemon yellow	25	entire
75	entire	25	entire
75	line plus	25	all but margin, faint
0	faint flush, flush at petal base	25	all but margin, faint
75	entire	50	line plus
25	line plus	75	all but margin
25	all but margin	25	all but margin

TABLE I (Continued)
Flat River, Missouri

% of petal red	distribution of red in calyx	% of petal red	distribution of red in calyx
50	line plus	25	all but margin
25	entire	75	dark, all but margin
25	line plus	50	all but margin
25	all but margin	25	all but margin
75	entire	75	entire
25	all but margin	50	entire
25	line plus	50	entire
25	entire	75	entire
25	entire, dark	75	entire, dark
50	all but margin	75	entire
25	entire, faint	75	entire
25	entire, faint	75	entire
25	all but margin	50	line plus
25	all but margin		

TABLE 2. Correlation in petal color (vertical scale) and sepal color (horizontal scale). Each dot represents the combination found in a single individual. Solid dots, Libertyville; open circles, Flat River.



between plants in any one locality and variation between one region and another are quite different processes. As far as *inter*-regional variation is concerned *H. virginiana* is perhaps quite as variable as *H. vernalis*.

FIGURE 1. Approximate distribution of *Hamamelis vernalis* (solid black) and *Hamamelis virginiana* (diagonal lines).

SUMMARY

Hamamelis vernalis, a species of very limited distribution, is shown to exhibit a high degree of *intra*-regional variability.

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NOTES ON THE GENUS PINUS

THE BLACK CONE OF PINUS PONDEROSA

GEORGE RUSSELL SHAW

IN SARGENT'S SILVA and in his Manual, also in Sudworth's Forest Trees of the Pacific Slope and in his Pine Trees of the Rocky Mountain Region, some of the cones of *Pinus ponderosa* Douglas are described as dark purple (nearly black) in color. The quotation below is copied from Sudworth in Bull. U. S. Dept. Agric. No. 460, p. 31 (1917).

"The cones of some trees are a bright grass-green when mature, while those of other trees are a dark purple, there being no essential difference between trees bearing cones so dissimilar in color."

In autumn when the cones are completely dried, the purple color disappears and is never found in herbarium collections. The ultimate color is brown, never purple or black. The explanation is simple. The purple color is a transient intermediate stage between the summer-green and the autumn-brown coloration of the maturing cones. Its life is brief and, as a consequence, it has escaped the notice of collectors. I have happened on it in five or six species, such as *Pinus rigida* Miller where the intermediate color is brown and the autumn-color is yellow, and also in *Pinus Greggii* Engelmann, where the intermediate color, strange to say, is a brilliant scarlet and the autumn-color is Naples yellow. The purpose of this article is to call attention to the intermediate color and to prevent further error from that source.

THE CAMBIUM AND ITS DERIVATIVE TISSUES
NO. VIII. STRUCTURE, DISTRIBUTION, AND DIAGNOSTIC SIGNIFICANCE OF VESTURED PITS IN DICOTYLEDONS

I. W. BAILEY

With four text figures and plates 61-63

INTRODUCTION

IN CONNECTION with an extended investigation of plasmodesma, I have had occasion to examine the cell walls and the pit membranes of a wide range of Gymnosperms and Angiosperms. It is evident that many of the structures in the tissues of the higher plants which are hypothesized as evidence for the existence of protoplasmic connections can not be interpreted as such.

The bordered pits in the vessels of the Leguminosæ and of certain other families of Dicotyledons are referred to as "sievelike" or "cribriform," a nomenclature based upon the assumption that the pit membranes are perforated by numerous small openings through which protoplasmic connections occurred in the immature vessel members. The sievelike appearance described by Dutailly (1), Jönsson (4), and others is not due to perforations of the pit membrane, but, as will be shown on the following pages, to minute outgrowths from the free surface of the secondary walls.

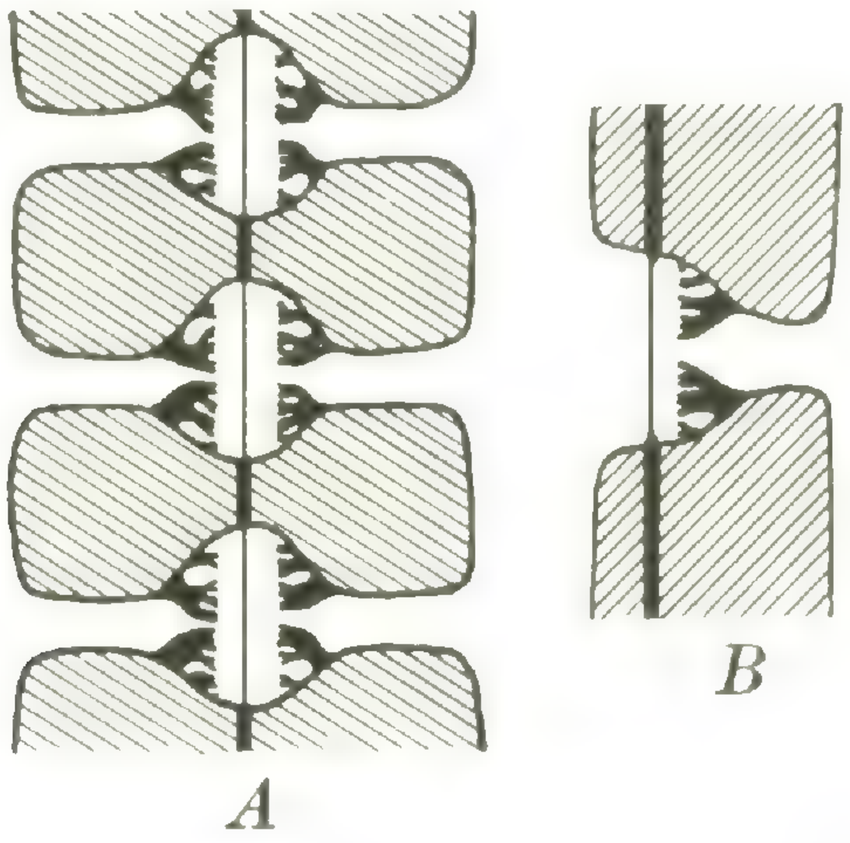
Although the so-called cribriform pits of tracheary tissue lose much of their putative physiological significance, they appear to be of considerable value both in the systematic study of woods and in discussions concerning the relationships and classification of specific groups of Dicotyledons.

STRUCTURE OF VESTURED (CRIBRIFORM) PITS

In unstained longitudinal sections of the wood of the Leguminosæ, Myrtaceæ, Polygonaceæ, Lythraceæ, Combretaceæ, and of a number of other representatives of the Dicotyledons, the bordered pits have, in surface view, a punctate appearance due to the presence of refractive processes of varying forms. In sections treated differentially with Haidenhain's hæmatoxylin and safranin, these processes are deeply stained and, in photomicrographs, appear as dark spots (*Plate 61, figs. 1, 3, 5,* and *7*) or as reticulate structures (*Figs. 2, 4,* and *6*) on a lighter colored background. By carefully focusing at successive levels it is

possible to demonstrate that there are two entirely independent sets of deeply-staining processes in each bordered pit-pair and that the punctate appearance of the bordered pits in surface view is not due to an unevenly thickened or perforated pit membrane.

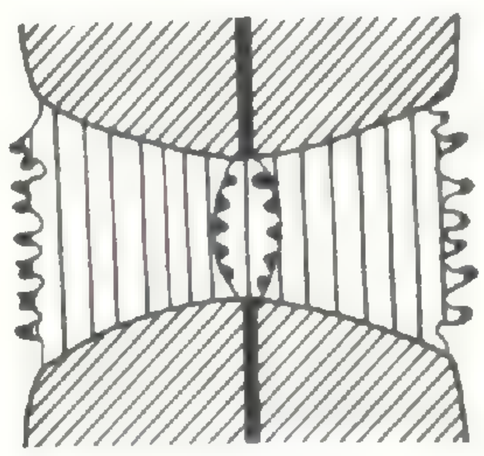
Owing to the small size of the pits and to the thickness of the walls in the tracheary elements of most Dicotyledons, the internal structural details of bordered and half-bordered pit-pairs may be observed most accurately in sectional rather than in surface views. It should be emphasized in this connection, however, that for this purpose extremely thin sections, 5-7 microns, are essential. The pits illustrated in *Plate 61, fig. 7* are shown in section in *Plate 62, fig. 10*. The thick, imperforate pit-membranes are in the median position, and the pit apertures and pit chambers are clearly visible. The dark-colored, toothlike processes obviously are attached to the overarching walls of the pit chambers and are not connected with the pit membranes. The more massive papillæ



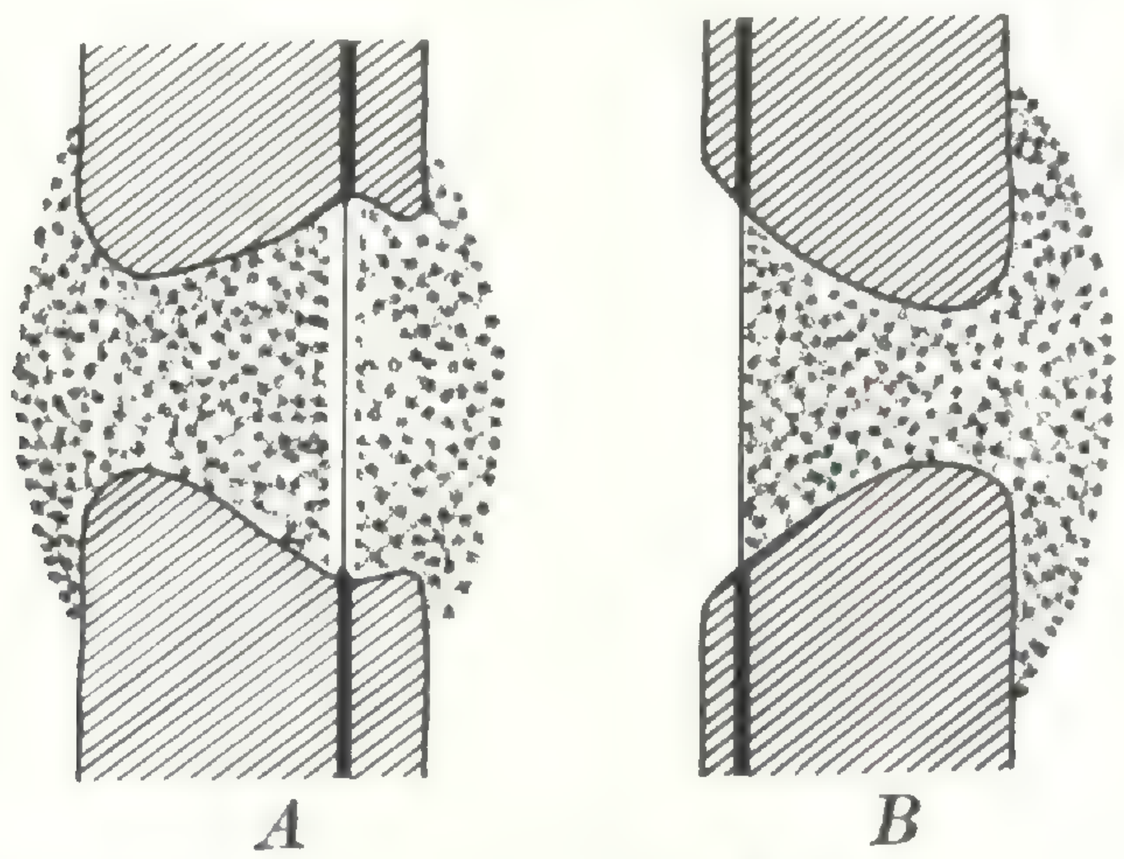
TEXT FIGURE 1. COMBRETUM SPECIES. (A) Sectional view of bordered pit-pairs in the walls of adjacent vessels, showing coralloid outgrowths from the overarching walls of the pit-chambers. (B) Sectional view of half-bordered pit-pair in the adjacent walls of a vessel (right) and of a parenchymatous element (left). The bordered pit is vestured, but the simple pit is not.

are attached close to the margins of the pit apertures and project diagonally towards the center of the pit chambers. Thus, in *Plate 61, fig. 7*, they are visible through the pit apertures, whereas the smaller peripheral papillæ, on the contrary, are partly obscured by the intervening portion of the secondary wall. *Plate 62, fig. 8* and *Text fig. 1A* are sections through the bordered pits illustrated in *Plate 61, fig. 5*. In these pits, as in the preceding ones, the deeply-stained processes are attached to the overarching walls and project into the pit chambers. They are characterized by having a distinctly branched or coralloid

structure, however. The pit membranes are shown in the median position in *Text fig. 1*, whereas in *Plate 62, fig. 8* they are rumpled and deflected to the right or left. A somewhat different type of structure is shown in *Plate 62, fig. 11*, a section through the bordered pits illustrated in *Plate 61, fig. 4*. Here the dark-colored processes form loose mats of branching and anastomosing filaments which are attached to the overarching walls of the pit chambers. Denser mats of finer texture which occlude the pit chambers are illustrated in *Plate 61, fig. 2* and *Plate 63, fig. 17*.



TEXT FIGURE 2. EUGENIA DICHOTOMA DC. Sectional view of bordered pit-pair in the walls of adjacent fiber-tracheids, showing papillary projections from the margins of both the inner and the outer apertures.

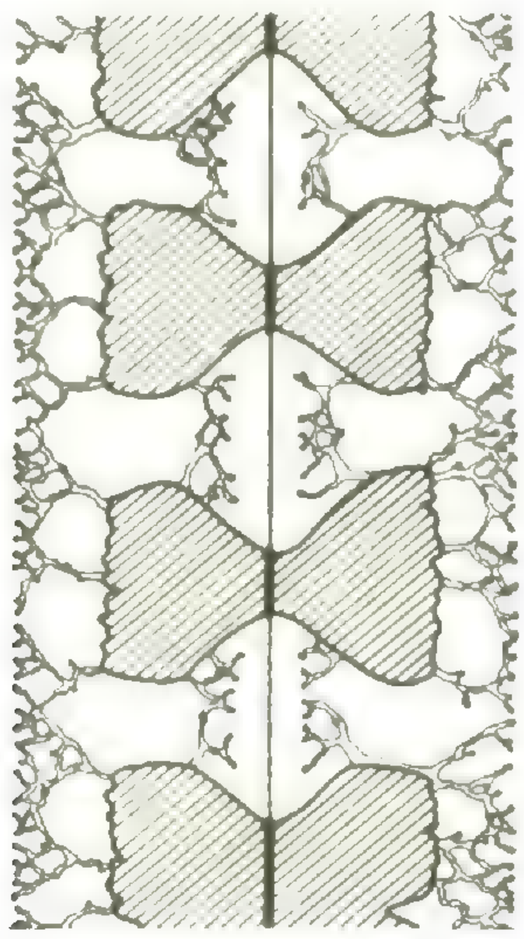


A *B*

TEXT FIGURE 3. PARASHOREA PLICATA Brandis. (A) Sectional view of bordered pit-pair in the adjacent walls of a vessel (left) and of a short tracheid (right). Mats of fine texture fill the entire pit-cavities and project more or less into the lumens of the cells. (B) Sectional view of half-bordered pit-pair in the adjacent walls of a vessel (right) and of a parenchymatous element (left). The bordered pit is vestured, but the simple pit is not.

The papillary, coralloid or filamentous processes are not confined to the pit chambers in all cases. As shown in *Plate 62, fig. 12* and *Text fig. 2*, they may be attached to the margins of both the inner and the outer apertures. They may fill the entire pit cavities and project more

or less into the lumens of the cells (*Plate 62, fig. 9* and *Text fig. 3A*). Not infrequently they occur on the inner surface of the secondary walls of the vessels (*Text fig. 4* and *Plate 63, fig. 16*) as well as within the bordered pits. They appear to be confined to tracheary elements, however. Thus, in half-bordered pit-pairs, they are present in the bordered pits of the tracheary elements but are absent in the simple pits of the adjoining parenchymatous cells (*Plate 62, fig. 13* and *Plate 63, fig. 18*, *Text figs. 1B* and *3B*).



TEXT FIGURE 4. VOCHYSIA HONDURENSIS Sprague. Sectional view of bordered pit-pairs in the walls of adjacent vessels, showing branched and anastomosing projections from the overarching walls of the pit-chambers and from the inner surfaces of the vessels.

In view of such facts as these, the terms *sievelike* or *cribriform* obviously should not be used in discussing the structures originally described by Dutailly and Jönsson. The terms *vestured pits* and *vestured walls* have been substituted for them by the Nomenclature Committee of the International Association of Wood Anatomists.

DEVELOPMENT OF VESTURED PITS

A priori, the structure of vestured pits and of vestured walls might be interpreted as due to adhering extraneous material deposited in mature tracheary cells during post mortem changes in the drying of sapwood or during the transformation of sapwood into heartwood. A study of living cells in sections of differentiating xylem reveals the fact that the curious processes in reality are formed by the cytoplasm during the later stages of the development of tracheary elements.

It should be noted in this connection, however, that punctate appearances (*Plate 63, fig. 15*) may be produced at times by extraneous or coagulated material which accumulates in the bordered pits during post

mortem changes and particularly during the transformation of sapwood into heartwood. True vestured pits may be distinguished from such artifacts by constancy of form and distribution, as well as by differential solubilities and other tests. In all the plants examined by me the vestured intervascular pits, when present in a species, occur in all vessels throughout a given specimen, in specimens from different parts of a tree, and in material from widely separated sources. The artifacts, on the contrary, are of extremely irregular and sporadic occurrence, not only in different specimens of a particular species, but even within the limits of a single section.

DISTRIBUTION OF VESTURED PITS IN DICOTYLEDONS

According to Record (6), pits with so-called cribriform membranes have been reported by Jönssen (4), Heiden (2), Ursprung (7), Moll and Janssonius (5), and Record as occurring in the secondary xylem of 20 families of Dicotyledons. In most cases, the number of species and genera listed is so restricted that it is not possible to formulate reliable conclusions concerning the occurrence and diagnostic value of vestured pits in specific groups of Dicotyledons. In 11 of the 20 families, the character is recorded in a single species or genus.

It seems advisable, accordingly, to tabulate my observations upon 2660 species, 979 genera, 152 families, and 33 orders. Families with vestured pits are printed in italics in Table 1. The numbers of genera and species investigated in each family are recorded in the columns at the right. Families in which "cribriform pits" have been reported by other investigators are marked with an asterisk.

TABLE I.

Orders and Families	Number of genera studied	Number of species studied
VERTICILLATAE		
Casuarinaceæ	1	8
PIPERALES		
Piperaceæ	2	3
Chloranthaceæ	1	1
SALICALES		
Salicaceæ	2	33
GARRYALES		
Garryaceæ	1	1
MYRICALES		
Myricaceæ	2	7

TABLE I. (Continued)

Orders and Families	Number of genera studied	Number of species studied
LEITNERIALES		
Leitneriaceæ	1	1
JUGLANDALES		
Juglandaceæ	5	24
FAGALES		
Betulaceæ	5	48
Fagaceæ*	7	107
URTICALES		
Ulmaceæ	6	33
Moraceæ	15	52
Urticaceæ	10	27
PROTEALES		
Proteaceæ	26	135
SANTALALES		
Santalaceæ	2	5
Olacaceæ	5	6
Octoknemataceæ	1	1
Loranthaceæ	2	2
ARISTOLOCHIALES		
Aristolochiaceæ	1	1
POLYGONALES		
*Polygonaceæ**	8	48
CENTROSPERMAE		
Chenopodiaceæ	5	8
Amarantaceæ	1	1
Nyctaginaceæ	2	6
Aizoaceæ	1	1
Caryophyllaceæ	1	1
RANALES		
Trochodendraceæ	3	4
Himantandraceæ	1	1
Cercidiphyllaceæ	1	1
Ranunculaceæ	4	4
Lardizabalaceæ	1	1
Berberidaceæ	1	3
Menispermaceæ	1	1
Magnoliaceæ	10	33
Annonaceæ	16	31

TABLE I. (Continued)

Orders and Families	Number of genera studied	Number of species studied
Myristicaceæ	4	6
Monimiaceæ	3	3
Lauraceæ*	19	47
Hernandiaceæ*	4	6
RHOEADALES		
Papaveraceæ	3	4
Capparidaceæ	*8*	*28*
Cruciferæ	*3*	*4*
Moringaceæ	1	2
ROSALES		
Saxifragaceæ	7	9
Pittosporaceæ	3	17
Brunelliaceæ	1	1
Cunoniaceæ	3	6
Hamamelidaceæ*	8	9
Eucommiaceæ	1	1
Platanaceæ	1	4
Crossosomataceæ	1	1
Rosaceæ*	24	77
Connaraceæ	4	8
*Leguminosæ**	*91*	*198*
Bauhinieæ	2	19
GERANIALES		
Oxalidaceæ	1	1
Linaceæ	1	1
Humiriaceæ	2	2
Erythroxylaceæ	1	4
Zygophyllaceæ	3	7
Rutaceæ	22	46
Simarubaceæ	9	16
Burseraceæ	7	15
Meliaceæ	18	56
Malpighiaceæ	*13*	*33*
*Vochysiaceæ**	*3*	*12*
Trigoniaceæ	1	5
Polygalaceæ	4	4
Dichapetalaceæ	1	1
Euphorbiaceæ*	35	55
Brideliæ	*2*	*6*

TABLE I. (Continued)

Orders and Families	Number of genera studied	Number of species studied
SAPINDALES		
Buxaceæ	1	4
Coriariaceæ	1	1
Anacardiaceæ	20	61
Cyrillaceæ	2	2
Aquifoliaceæ	3	12
Celastraceæ	23	53
Salvadoraceæ	1	1
Staphyleaceæ	2	4
Icacinaceæ	6	7
Aceraceæ	1	25
Hippocastanaceæ*	1	6
Sapindaceæ	26	48
Sabiaceæ	2	3
RHAMNALES		
Rhamnaceæ*	9	24
Vitaceæ	3	7
MALVALES		
Elaeocarpaceæ	4	15
Tiliaceæ	12	24
Malvaceæ	9	11
Bombacaceæ	5	8
Sterculiaceæ	9	31
Scytopetalaceæ	1	1
PARIETALES		
Dilleniaceæ	4	14
Eucryphiaceæ	1	3
Ochnaceæ-Exalbuminosæ	5	21
Albuminosæ	2	4
Caryocaraceæ	1	1
Marcgraviaceæ	1	2
Theaceæ	8	11
Guttiferæ*	17	25
Dipterocarpaceæ	8	32
Fouquieraceæ	1	1
Cistaceæ	1	1
Winteranaceæ	1	1
Violaceæ	4	5

TABLE I. (Continued)

Orders and Families	Number of genera studied	Number of species studied
Flacourtiaceæ	15	31
Stachyuraceæ	1	1
Passifloraceæ	3	4
OPUNTIALES		
Cactaceæ	2	7
MYRTIFLORAE		
Oliniaceæ	1	2
Thymelæaceæ	3	3
Elæagnaceæ	3	6
*Lythraceæ**	3	10
*Sonneratiaceæ**	1	2
*Blattiaceæ**	1	2
*Crypteroniaceæ**	1	2
Punicaceæ	1	1
Lecythidaceæ	18	45
Rhizophoraceæ	13	32
Nyssaceæ	1	4
Alangiaceæ	1	1
*Combretaceæ**	7	24
*Myrtaceæ**	15	89
*Melastomataceæ**	4	9
*Oenotheraceæ**	1	3
UMBELLIFLORAE		
Araliaceæ*	25	66
Umbelliferæ	2	2
Cornaceæ*	7	15
ERICALES		
Clethraceæ	1	3
Ericaceæ	8	24
Epacridaceæ	3	9
PRIMULALES		
Myrsinaceæ	8	19
EBENALES		
Sapotaceæ	11	49
Ebenaceæ	2	27
Symplocaceæ	2	6
Styracaceæ	4	8

TABLE I. (Continued)

Orders and Families	Number of genera studied	Number of species studied
CONTORTAE		
Oleaceæ*	16	59
Nathusia	1	1
Forestiera	1	5
Loganiaceæ	6	7
Apocynaceæ	15	29
*Asclepiadaceæ**	1	2
TUBIFLORAE		
Convolvulaceæ	1	2
Polemoniaceæ	1	2
Hydrophyllaceæ	1	1
Boraginaceæ	5	19
Verbenaceæ	12	29
Labiatæ	4	6
Solanaceæ	6	11
Scrophulariaceæ*	6	9
Bignoniaceæ	11	25
Gesneriaceæ	1	1
Myoporaceæ	2	3
RUBIALES		
*Rubiaceæ**	41	78
Caprifoliaceæ*	5	18
Dipsacaceæ	2	2
CUCURBITALES		
Cucurbitaceæ	1	2
CAMPANULATAE		
Compositæ*	12	21

DIAGNOSTIC VALUE OF VESTURED PITS

In the material examined by me, true vestured pits are either present throughout the secondary xylem of a species or genus or are entirely absent. A similar constancy in the presence or absence of these structures appears to prevail in most families of Dicotyledons. In only four of the 152 families tabulated in Table 1, have I encountered vestured pits in certain representatives of a family and not in others. It should be noted in this connection, however, that in three of the four families the distribution of vestured pits correlates with major subdivisions. Thus, in the Leguminosæ, the vestured pits are present in all the species

and genera examined, with the exception of the Bauhinieæ, whereas in the Euphorbiaceæ they are absent except in the Bridelieæ. They are present in the Exalbuminosæ of the Ochnaceæ, but appear to be absent in the Albuminosæ. In other words, the Oleaceæ, are the only family in which the distribution of vestured pits fails to correlate closely with the systematic classification.

It may be objected that cribriform structures have been reported in representatives of 13 families which are not italicized by me. These families are listed in Table 2.

TABLE 2

Families	Genera and species	Reported by
Fagaceæ	Quercus alba L.	Jönsson (4)
	Q. Cerris L.	"
	Q. pedunculata Ehrh.	"
	Q. obtusiloba Michx.	"
Lauraceæ	Numerous genera and species	Janssonius (3)
Hernandiaceæ	Hernandia peltata Meisn.	"
Hamamelidaceæ	Altingia excelsa Nor.	Moll & Janssonius (5)
Rosaceæ	Cerasus serotinus hort.	Jönsson (4)
	Prunus brigantiaca Vill.	"
Hippocastanaceæ	Aesculus Hippocastanum L.	"
	A. rubicunda hort.	"
Rhamnaceæ	Phylica ericoides L.	"
Guttiferæ	Calophyllum Inophyllum L.'	Ursprung (7)
	C. Calaba Jacq.	Record (6)
Araliaceæ	Hedera helix L.	Jönsson (4)
Cornaceæ	Mastixia trichomata Blume	Moll & Janssonius (5)
Scrophulariaceæ	Veronica Andersoni hort.	Jönsson (4)
Caprifoliaceæ	Viburnum sundaicum Miq.	Janssonius (3)
Compositæ	Helichrysum moniliferum hort.	"

Obviously it is essential to determine whether the "sievelike" appearances reported by Jönsson and others are due to the presence of vestured pits or to artifacts such as are produced during post mortem changes or during the transformation of sapwood into heartwood.

As indicated in Table 1, true vestured pits do not occur in any of the 107 species of Fagaceæ that I have studied. Nor have I succeeded

in finding them in any of the numerous specimens of *Quercus alba*, *Q. Cerris*, *Q. obtusiloba*, or *Q. robur* L. (*Q. pedunculata* Ehrh.) that I have investigated. Not only are vestured pits entirely absent in 19 genera and 47 species of the Lauraceæ and in 77 species and 24 genera of the Rosaceæ, but also in numerous specimens of the Prunoideæ, i. e., *Prunus* and its subgenera *Prunophora*, *Amygdalus*, *Cerasus*, and *Padus*. Similarly, I have been unable to find vestured pits in *Altingia*, the Bucklandieæ, Altingieæ, Parrotieæ, and Hamamelideæ of the Hamamelidaceæ; in *Aesculus Hippocastanum*, and five other species of the Hippocastanaceæ; in the Zizypheæ and Rhamneæ of the Rhamnaceæ; in *Calophyllum Inophyllum* and other Calophylloideæ of the Guttiferæ; in *Hedera helix* and 65 other representatives of the subgroups, Scheffleræ and Aralieæ, of the Araliaceæ; or in the Mastixioideæ and Cornoideæ of the Cornaceæ.

Although I have failed to find true vestured pits in any of the families listed in Table 2, I have frequently encountered artifacts of various types. In such genera as *Quercus*, *Altingia*, *Calophyllum*, *Hedera*, *Mastixia*, etc., these artifacts may produce a punctate appearance which closely simulates that of vestured pits. Therefore, in view of the fact that punctate appearances were interpreted as evidence of a sievelike structure, it is not surprising that the species in Table 2 were recorded as having pits with cribriform membranes.

It should not be inferred from this that more detailed and extensive surveys may not reveal the presence of vestured pits in additional families of Dicotyledons. The vestured condition appears to have arisen independently a number of times. To assume that all plants with vestured pits are closely related or are derived from common ancestors which possessed such structures leads to a *reductio ad absurdum*. Vestured pits occur in the more highly specialized types of tracheary tissue and are absent in plants which have a primitive combination of structural characters in the xylem. If vestured pits have arisen independently a number of times, it is not unlikely that genera may ultimately be found in which these structures are present in certain species and are absent in others. It may be inferred, however, from a statistical analysis of Table 1, that sporadic distributions of vestured pits are likely to be of relatively infrequent occurrence in the case of subgenera, genera, and subfamilies.

That vestured pits are extremely useful diagnostic criteria in the systematic study of woods was clearly demonstrated during the course of the present investigation. Most collections of wood specimens, even when accompanied by herbarium specimens, contain a varying number of errors. In other words, the fact that an herbarium specimen and a

sample of wood bear the same number is unfortunately no guarantee that both specimens came from the same tree or that the herbarium specimen was correctly identified. Collections of woods commonly pass through a number of hands and may be subdivided, renumbered, or relabeled. Furthermore, transpositions are likely to occur during the preparation of microscopic slides unless a painstaking system of checking and rechecking is employed. In my reconnaissance of Dicotyledons, I encountered true vestured pits in slides of putative representatives of a number of families which are not italicized in Table 1, and, conversely, ordinary bordered pits in families which are italicized. In all these families, with the exception of the Oleaceæ, the aberrant specimens proved to be errors which could thus be eliminated from the collections.

Similar combinations of anatomical characters occur not infrequently in the woods of families which are widely separated in the systematic classification of Dicotyledons. For example, the secondary xylem of the Osage Orange, *Maclura pomifera* (Raf.) Schneid., so closely resembles that of the Black Locust, *Robinia pseudoacacia* L., that it is extremely difficult to distinguish the woods by anatomical criteria. The woods of the two genera may be identified with certainty, however, by the fact that vestured pits occur in *Robinia* and are entirely absent in *Maclura*.

As indicated in the accompanying text figures and photomicrographs, the number, size, and form of the refractive processes vary considerably in different plants. In certain species and genera the processes are confined to the intervascular pits, whereas in others they may occur as well in the half-bordered pit-pairs, in the fiber tracheids, or upon the inner surfaces of the tracheary walls. Such differences may obviously be utilized as diagnostic criteria in the systematic study and classification of woods.

SUMMARY AND CONCLUSIONS

1. Many of the structures in the tissues of the higher plants which are hypothesized as evidence for the existence of protoplasmic connections can not be interpreted as such.
2. The so-called sievelike appearance of the pits in the vessels of Leguminosæ and of other families of Dicotyledons is not due to perforations of the pit membranes, but to minute outgrowths from the free surfaces of the secondary walls.
3. These refractive processes which vary considerably in size, number, and form are not confined to the pit-chambers in all cases, but may occur on the inner surface of the secondary walls of the vessels.

4. They appear to be restricted to tracheary elements; in half-bordered pit-pairs they are present in the bordered pits of the tracheary elements, but are absent in the simple pits of the adjoining parenchymatous cells.
5. Pits which have these refractive processes may be referred to as *vestured*.
6. In the Dicotyledons (2660 species and 979 genera) examined by me, vestured pits are either present throughout the secondary xylem of a species or genus or are entirely absent. A similar constancy in the presence or absence of these structures appears to prevail in most subfamilies and families.
7. Vestured pits, therefore, are of considerable value both in the systematic study of woods and in discussions concerning the relationships and classification of specific groups of Dicotyledons.

ACKNOWLEDGMENTS

The text-figures used in this paper were drawn by my assistant, Mrs. Ernest C. Abbe. I am much indebted to Professor S. J. Record for his kindness in providing woods of various families of Dicotyledons.

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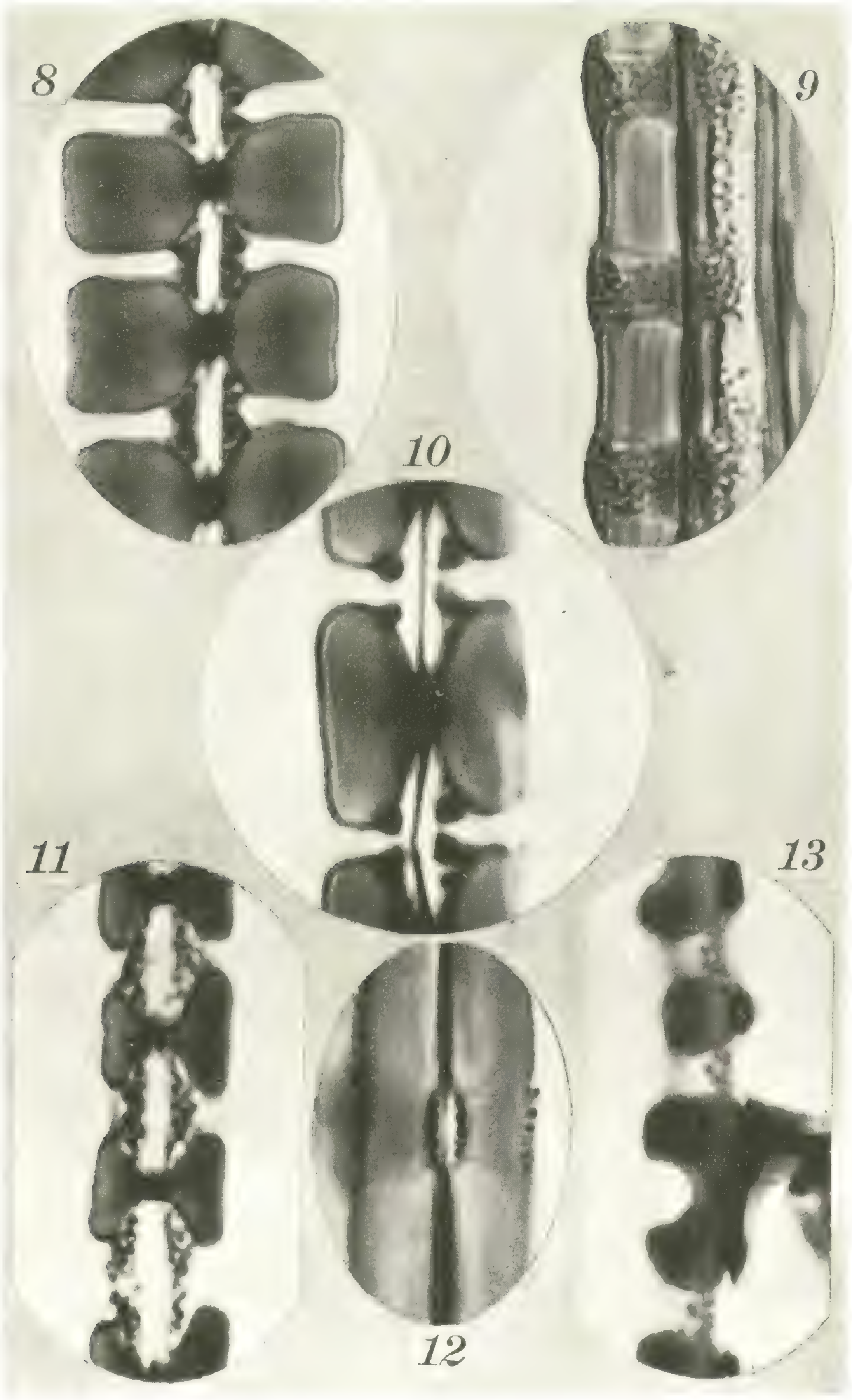
DESCRIPTION OF PLATES

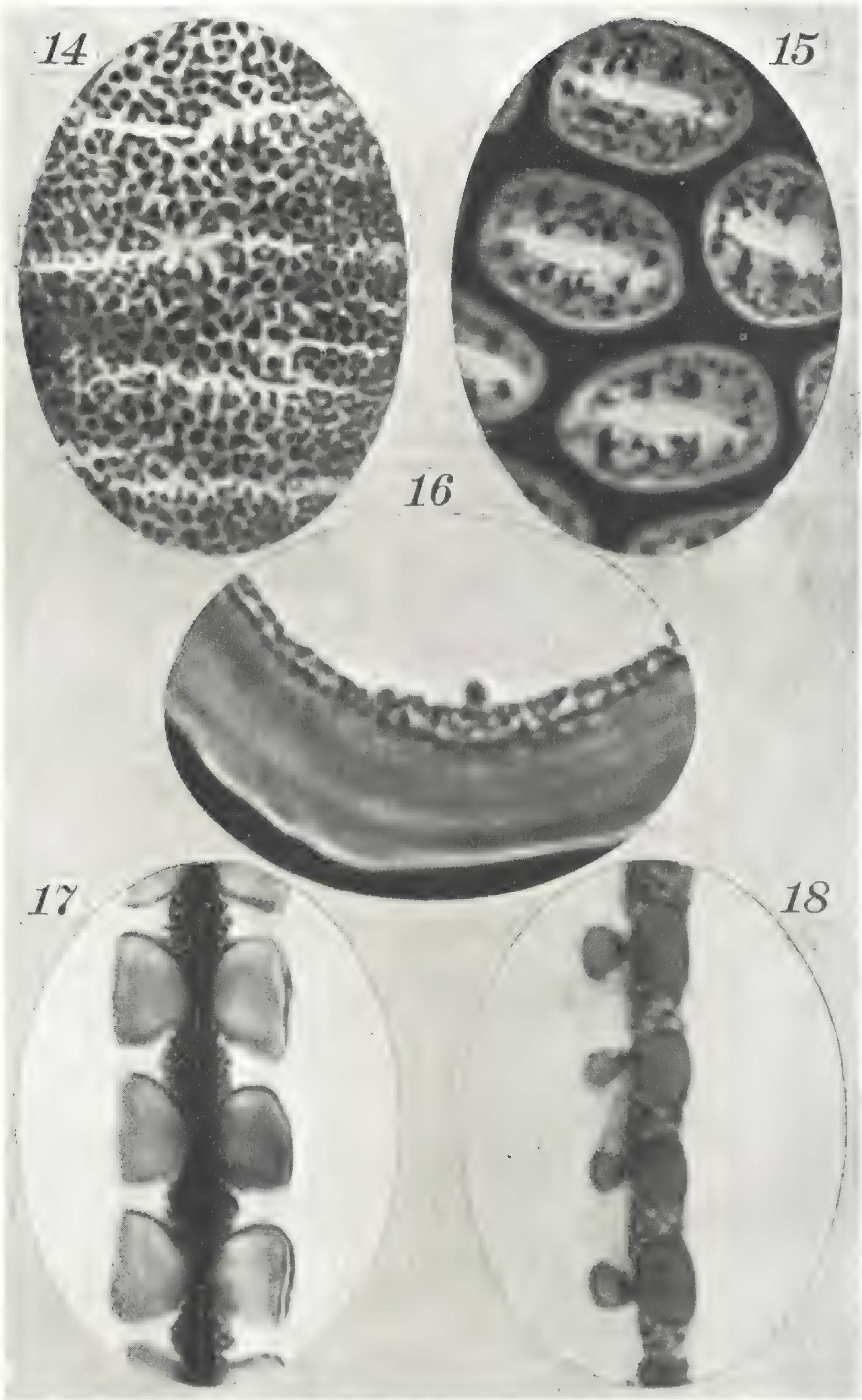
PLATE 61

Fig. 1. *Prosopis juliflora* DC. Surface view of bordered pits, showing toothed appearance of pit apertures. × 2500.
Fig. 2. *Duabanga moluccana* Blume. Surface view of bordered pits, showing finely punctate appearance. × 1900.
Fig. 3. *Fuchsia Colensoi* Hook. f. Surface view of bordered pits, showing toothed appearance of elongated pit apertures. × 2500.
Fig. 4. *Tibouchina mutabilis* Cogn. Surface view of bordered pits, showing mats of branching and anastomosing processes. × 2500.
Fig. 5. *Combretum specics*. Surface view of bordered pits, showing coarsely punctate appearance. × 2500.

VESTURED PITS IN DICOTYLEDONS



VESTURED PITS IN DICOTYLEDONS



VESTURED PITS IN DICOTYLEDONS

Fig. 6. *Eugenia alternifolia* Wight. Surface view of half-bordered pit-pair, showing irregularly punctate appearance. × 2500.
Fig. 7. *Terminalia Chebula* Retz. Surface view of bordered pits, showing coarsely toothed appearance of pit apertures. × 2500.

PLATE 62

Fig. 8. *Combretum species.* Sectional view of pits illustrated in *Fig. 5,* showing coralloid outgrowths from the overarching walls of the pit chambers. × 2500.
Fig. 9. *Parashorea plicata* Brandis. Sectional view of three pairs of bordered pits in the adjacent walls of a vessel (left) and a short tracheid (right). Mats of fine texture fill the entire pit cavities and project more or less into the lumens of the cells. × 2700.
Fig. 10. *Terminalia chebula* Retz. Sectional view of pits illustrated in *Fig. 7,* showing massive projections from the overarching walls of the pit chambers. The imperforate membranes are in the median position. × 3300.
Fig. 11. *Tibouchina mutabilis* Cogn. Sectional view of pits illustrated in *Fig. 4,* showing attachment of mats to overarching walls of the pit chambers. × 2500.
Fig. 12. *Eugenia dichotoma* DC. Sectional view of pit-pair in the adjacent walls of fiber-tracheids, showing toothed inner and outer apertures. × 2500.
Fig. 13. *Parkinsonia Torreyana* S. Wats. Sectional view of half-bordered pit-pairs in the adjacent walls of a vessel (left) and parenchyma (right). The bordered pits are vestured, but the simple pits are not. × 2500.

PLATE 63

Fig. 14. *Bridelia retusa* Spreng. Surface view of vestured inner surface of vessel. × 2500.
Fig. 15. *Sciadodendron excelsum* Griseb. Surface view of bordered pits, showing artifacts which simulate the deeply-staining processes of vestured pits. × 2500.
Fig. 16. *Acacia flexicaulis* Benth. Sectional view of wall of vessel, showing vestured inner surface. × 2500.
Fig. 17. *Duabanga moluccana* Bl. Sectional view of pits illustrated in *Fig. 2,* showing mats of fine texture within the pit chambers. × 1900.
Fig. 18. *Eugenia alternifolia* Wight. Sectional view of half-bordered pit-pairs in the adjacent walls of a vessel (right) and parenchyma (left). The bordered pits are vestured, but the simple pits are not. × 2500.

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SPECIES HYBRIDS IN PLATANUS AND CAMPSIS

KARL SAX

With two text figures

ACCORDING TO SEWARD (1931), *Platanus* is one of the oldest of broad-leaved trees, and fossil types have been found which are very similar to the fertile branches of modern species. This genus was one of the most widely spread of the earlier cretaceous dicotyledons. The cretaceous *Platanus* was more variable and had a much wider distribution than the existing species.

The genus *Platanus* is now found in North America to Mexico and from southeastern Europe to India (Rehder, 1927). The American species, *P. occidentalis*, is hardier than the Old World species, *P. orientalis;* but the two species must be rather closely related because they have produced the fertile hybrid *P. acerifolia*. According to Henry and Flood (1919), *P. acerifolia* originated as a natural hybrid between the Occidental and Oriental Plane. It probably originated in the Oxford Botanic Garden about 1670. The hybrid is intermediate in leaf and fruit characters and seems to possess unusual vigor. The hybrid is extensively used for planting in the streets of European towns where neither of the parental species will survive. Seedlings from the hybrid are variable, some of them resembling the parental species.

The fact that the species hybrid is fertile, and segregates in the second generation, would indicate that the parental species have the same number of chromosomes and that their chromosomes are similar. The haploid chromosome number of *Platanus* has been reported to be 21 (Winge, 1917), 8 (Brouwer, 1924), and 20-22 (Bretzler, 1924). Permanent smear preparations of pollen mother cells from *P. occidentalis* and *P. acerifolia* show clearly that the number of chromosomes is 21, as reported by Winge. The chromosomes are paired regularly at the first meiotic division, and there is no evidence of lagging chromosomes at any stage in the meiotic divisions. The chromosomes at the first meiotic metaphase, and at telophase, in the hybrid, are represented in Figure 1. One of the chromosomes is somewhat smaller than the others, especially when fixed in aceto-carmine solution. The chromosomes are too small and numerous to permit an accurate determination of chiasma frequency, but both rod and ring chromosomes were observed. The average chiasma frequency is about 1.5 per bivalent. About 90 percent of the pollen is good in the hybrid.

The apparent compatibility of the chromosomes from the two parental species in the first generation hybrid indicates that the Old and New World species have undergone no very fundamental changes since their segregation and differentiation. Although this genus has undergone no very fundamental changes for a long period of time, and even though it is one of the oldest dicotyledons so far discovered, it does not possess characters which mark it as a primitive type or as an early member of an evolutionary series (Seward, 1931). It is of interest that *Platanus* is the only genus of the family Platanceæ.

Another valuable hybrid between species from the Old and New World is *Campsis Tagliabuana* (*C. hybrida*) (Rehder, 1932). The parental species are *C. radicans*, from central and southern United States, and *C. chinensis*, from China. The American species seems to

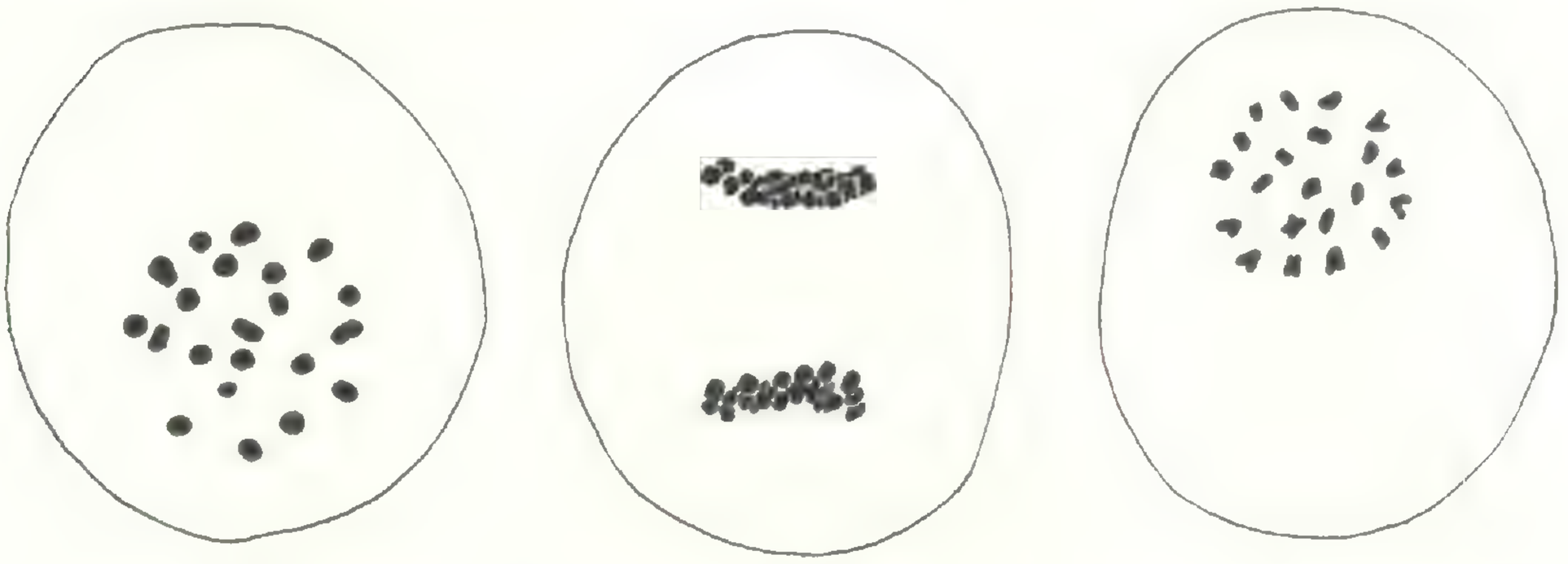

FIGURE 1. Meiotic chromosomes of *Platanus acerifolia* at metaphase and telophase. The 21 bivalents divide regularly. × 2000.

be hardier and more vigorous than the Chinese species, but the flowers of *C. chinensis* are more attractive. The hybrid has the good qualities of both parents. It is almost as attractive as the Chinese species and has the hardiness of the American species. The hybrid forms are grown in many gardens of southern Massachusetts (Anderson, 1933).

The hybrid was first recorded by Visiani as having been raised by the brothers Tagliabue apparently some time before 1859, but doubtless it has originated independently elsewhere afterwards or even before 1859. Natural hybrids have also been obtained at the Botanical Garden in Washington, D. C. A more complete account of the origin and characteristics of *Campsis Tagliabuana* has been presented in the Arnold Arboretum bulletin of popular information, by Edgar Anderson.

The hybrids are partially fertile, and numerous segregates have been produced. The chromosome number is undoubtedly the same for both the parental species and the hybrid. There are 20 pairs of small chromosomes at the first meiotic division in *C. radicans*. No representa-

tives of *C. chinensis* were available for study, but a hybrid segregate much like the Chinese species, also has 20 pairs of chromosomes. The hybrid has 20 pairs of chromosomes which are perfectly regular in pairing and disjunction at meiosis. The chromosome number found in the hybrid is in accord with the count reported by de Vilmorin and Simonet (1927). The chromosomes are very small and usually form rod bivalents at the first meiotic division. The chiasma frequency is apparently little more than one per bivalent.

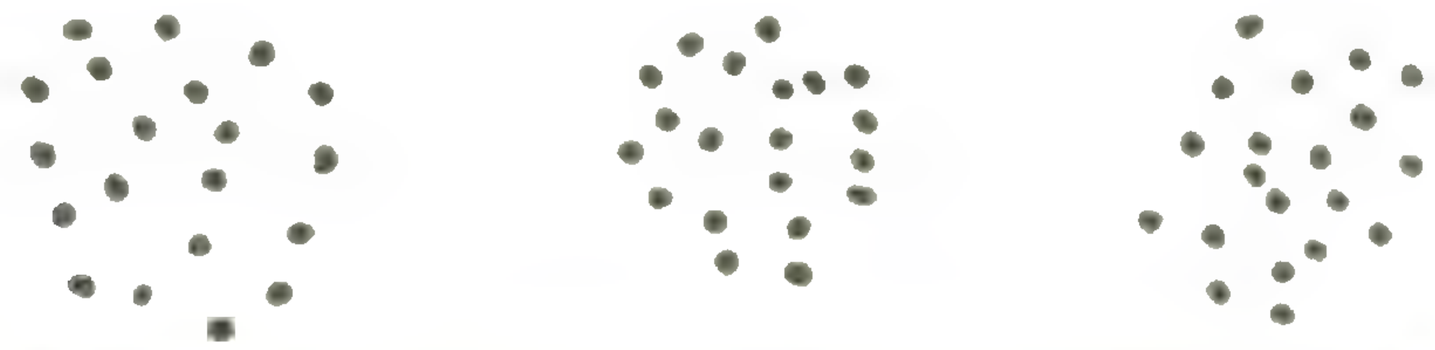

FIGURE 2. Meiotic chromosomes of *Campsis radicans*, *C. Tagliabuana* and *C.* spec. (probably a hybrid segregate resembling *C. chinensis*). The divisions are regular in the one parental species examined and in the hybrids. × 2000.

Although there is regular pairing and disjunction of the chromosomes in *Campsis Tagliabuana*, about 50 per cent of the pollen is sterile. There is less than 5 per cent pollen sterility in *C. radicans*. Pollen sterility in species hybrids which have regular meiotic divisions is also found in other genera (*Primula kewensis*, diploid, Newton and Pellew, 1929; *Deutzia* and *Philadelphus*, Sax, 1931; *Tradescantia*, Sax and Anderson, 1933). Pollen sterility in such species hybrids might be caused by unequal interchange of chromosome segments in one of the parental species. The interchange of segments between non-homologous chromosomes has been found in a considerable number of different genera in slightly related families, and may be much more extensive than is indicated by the formation of rings or chains of chromosomes. In a species with a low chiasma frequency, segmental interchange would not result in chromosome rings, and if segmental interchange were unequal, chromosome pairing would be regular in both heterozygous and homozygous forms. The heterozygous types will be eliminated unless they possess a much greater survival value to compensate for their partial sterility or unless balanced lethals are involved. As a result, plants should originate which are homozygous for segmental interchange chromosomes, as is known to be the case in *Datura*, *Pisum*, and *Oenothera*.

If an individual homozygous for two segmental interchange chromosomes (the minimum number possible) is crossed with a normal plant, the chromosomes should pair as bivalents and divide normally if the

segmental interchange is unequal and if the chiasma frequency is low. But the random segregation of the chromosomes would result in 50 per cent non-disjunction of a chromosome segment. If both segments are essential for gametophyte development, the pollen sterility would be 50 per cent; if only the longer segment is essential, the pollen sterility would be 25 per cent. Pollen sterility would be almost complete in a plant heterozygous for four or five segmental interchange chromosomes.

Segmental interchange might well be one of the factors involved in the differentiation of species. A form with relatively few segmental interchange chromosomes would tend to be isolated from the normal type because of the sterility of the F_1 hybrid between the two forms. Unless lethal factors are associated with the segmental interchange chromosomes, the homozygous forms should have a higher survival value. Variations originating in the different lines homozygous for chromosome structure would not be swamped by intercrossing and would tend to be developed more or less independently in different structural genoms.

SUMMARY

Platanus acerifolia, a natural hybrid between *P. orientalis* of southeastern Europe and *P. occidentalis* of North America, has 21 pairs of chromosomes which pair regularly at meiosis. The hybrid is fertile, and genetic segregation occurs in the second generation. These facts indicate that the parental genoms are similar and are compatible with each other, even though the parental species have been isolated for a long period of time.

Campsis Tagliabuana, a natural hybrid between *C. chinensis* from China and *C. radicans* from North America, has 20 pairs of chromosomes which pair regularly at meiosis. Although the reduction divisions are regular, there is about 50 per cent pollen sterility in the F_1 hybrid. The association of regular chromosome pairing and partial or nearly complete pollen sterility in species hybrids may be the result of segmental interchange between non-homologous chromosomes in one or both parental species.

The species hybrids in the above genera contain the desirable characters of the parental species and are especially valuable because of their hardiness. Such hybrids are good illustrations of what may be expected from many hybrids between Old and New World species.

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CHROMOSOME BEHAVIOR IN CALYCANTHUS

KARL SAX

With four text figures

TWO SPECIES of *Calycanthus* are found in eastern United States. *Calycanthus floridus* L. is found from Virginia to Florida while *C. fertilis* extends from Pennsylvania to Georgia and Alabama (Rehder, 1927). Herbarium material in the Arnold Arboretum includes *C. fertilis* from eleven localities in North and South Carolina and Georgia, and *C. floridus* from fifteen localities in South Carolina, Georgia and Alabama. The ranges of the two species overlap to some extent, but there is little evidence of hybridization although the two species are similar, differing chiefly in leaf characters. Varieties of the two species have been described, but in the case of *C. floridus* at least, the variety (*ovatus*) is rare and is apparently known only in cultivation, and appears to be of European garden origin.

Chromosome counts were obtained from one plant of *C. fertilis*, two plants of *C. floridus*, and two varieties of these species. In both species there are eleven pairs of chromosomes at meiosis. The homologous chromosomes are united by terminal or subterminal chiasmata. The chiasma frequency is somewhat less than two per bivalent at early metaphase. Some of the chromosomes are apparently heterobrachial and the separation of the short arms at late metaphase and early anaphase gives the impression of a prevalence of rod bivalents at the later stages although at early metaphase most of the chromosomes are ring bivalents. The chromosomes of *C. fertilis* are shown at early metaphase (Fig. 1) and those of *C. floridus* are shown at late metaphase of the first meiotic division (Fig. 2).

A variety of *C. fertilis* also had eleven pairs of chromosomes which pair and divide regularly at meiosis. The other variety in the Arboretum, *C. floridus ovatus*, is a triploid. At meiosis there are often eleven trivalents, although from one to four univalents are usually found. The trivalents are found in the form of chains, rings and rods, and Y's (Fig. 3). There is some irregularity in the first meiotic division, including both trivalents and univalents, and the chromosomes are distributed irregularly to the poles. The chromosomes at second metaphase following a comparatively regular division are shown in Figure 4. In one case a distribution of 20-13 was observed, but as a rule the numbers of chromosomes passing to each pole are approxi-

mately equal. Occasionally more than thirty-three chromosomes are found at the two poles, due presumably to a precocious division of one or more univalents.

The pollen sterility of the triploid is about fifty per cent as compared with about five per cent in each of the pure species.

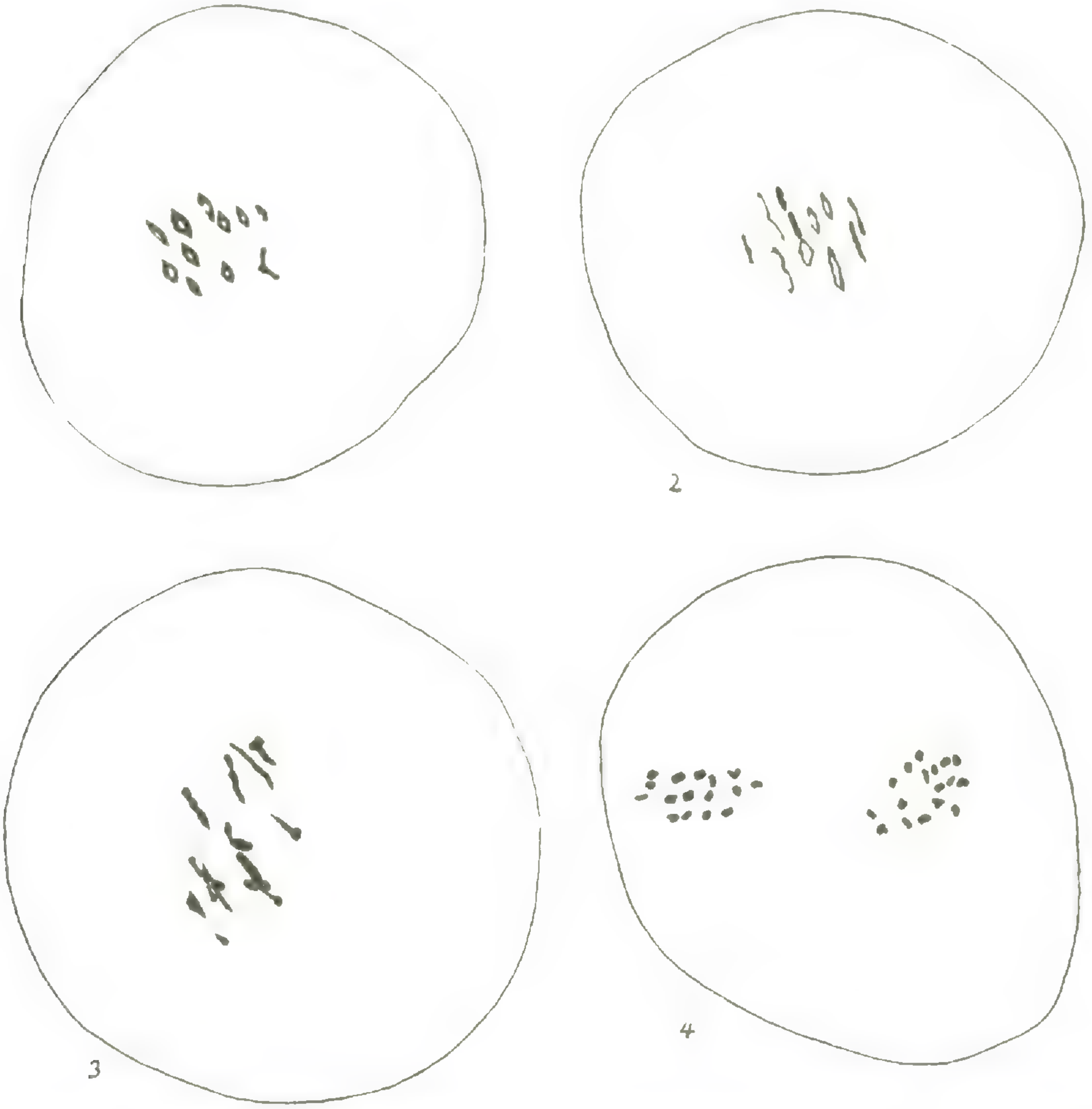

Figure 1. CALYCANTHUS FERTILIS: chromosomes at early metaphase of the first meiotic division.—Figure 2. CALYCANTHUS FLORIDUS: late metaphase.—Figure 3. CALYCANTHUS FLORIDUS OVATUS: a triploid showing trivalent chromosomes at meiosis.—Figure 4. The triploid variety showing chromosome distribution at the second meiotic division.—The figures were drawn from aceto-carmine preparations. Magnification × 1200.

Since the two species of *Calycanthus* are similar in taxonomic characters and overlap in their distributions, the occurrence of natural hybrids might be expected. Some of the diploid varieties may be of hybrid origin, but there seems to be no extensive hybridization, and the two species are rather well differentiated. It is possible that tetra-

ploid forms of these species exist and that the triploid variety is a hybrid between a tetraploid, *C. floridus* and a diploid *C. fertilis*, but it seems more probable that the *ovatus* variety is an autotriploid. The two plants of *C. floridus* in the Arboretum are typical for the species and both are diploids. The variety *ovatus* originated, or was first found, in a European garden and is not known to occur in nature. No species of *Calycanthus* is a native of Europe.

Chromosome irregularity and pollen sterility have been considered as evidence of hybridity. In the case of *Calycanthus floridus ovatus* it is improbable that chromosome irregularity and pollen sterility can be attributed to species hybridization. An autotriploid originating within a species would be expected to show the chromosome irregularities and pollen sterility. Chromosome irregularity at meiosis and pollen sterility can also be caused by segmental interchange with absolutely no change in the taxonomic characters of the plants involved. *Tradescantia edwardsiana*, for example, is a well marked species. The occasional segmental interchange plants show about fifty per cent pollen sterility, although they are taxonomically the same as the normal fertile plants (Sax & Anderson 1933).

Chromosome irregularities may also be caused by variations in temperature and by genetic factors. On the other hand species hybrids often show regular chromosome pairing and division. In some of these hybrids there is much pollen sterility, but others are relatively fertile. Undoubtedly wide species crosses often result in hybrids which exhibit irregular chromosome behavior at meiosis, but chromosome irregularity is not necessarily evidence of species hybridization.

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ORIGIN AND BEHAVIOR OF THE NUCLEOLUS IN PLANTS

HAIG DERMEN

With plates 64 to 67

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A. INTRODUCTION

THE PROBLEM of the nucleolus is one which has attracted investigators for many years, but despite the large amount of work done on this nuclear body, the problem is far from solved. The origin, the development, the relation between nucleolus and chromosomes, and the nucleolar function are phases of the problem which have received attention, but there is little concerted opinion regarding them. That the nucleolus is an essential component of cells is evidenced by the fact that it is present, very probably, in all types of cells of all organisms, a fact which may be responsible for Wilson's question (1925) "whether the nucleolus may not play a more active and important part in cell metabolism than most writers have hitherto assumed."

The importance of a cell body is not necessarily dependent upon its continuity and permanence in a particular structural form, such as is characteristic of the chromosomes. Unlike the chromosomes the nucleolus possesses no constant structural form and is not even continuous, in the strict sense of the word, appearing and disappearing during both mitosis and meiosis. This peculiarity alone has perhaps caused a confusion in evaluating its significance, but when its universality is considered, it would appear that its function is one of vital import to the cell, and its appearance and disappearance in the cell cycle may be correlated with that function, which is probably of a metabolic nature.

The present work is primarily intended to determine the origin and to trace the development of the nucleolus through all the stages of mitotic and meiotic development of the nucleus in plants of diverse types and to see what relationship exists between the chromosomes and nucleolus.

Many of the recent investigations are concerned with the origin of the nucleolus and its relation to the chromosomes. Its function has been, and still is, a matter of speculation. Van Camp (1924) believes that nucleoli originate from the chromosomes at telophase in the form of small globules which later fuse into larger forms, but still later, during the development of the chromosomes, through a direct contact established between a continuous spireme and the nucleolus, the nucleolar substance flows back again over the chromosomes to be again dissociated during telophase. Zirkle (1928 and 1930), by developing specific fixatives, confirms the idea of direct transportation of nucleolar substance into the spireme and demonstrates the presence of this substance in a skeleton form in the anaphase chromosomes. Heitz (1931a and 1931b) offers evidence to show that the nucleolus originates from the satellite chromosomes and only from them, and formulates a theory

that accounts for the symmetry of the nucleoli in number, size, position, and form in the telophase of the sister* (daughter) nuclei. Abele (1930) seems to believe that the nucleolar substance divides equally, as do the chromosomes, thus giving rise to nucleoli in sister nuclei that show symmetry in all respects and that "jeder Nukleolus hat seinen Partner in der anderen Tochterzelle."

As for the function of the nucleolus, Montgomery (1898) assumes that the nucleolar substance is connected with the nutritive processes of the cell and, as an assimilation organ, takes food from the cytoplasm. Meyer (Guillermond et al., 1933) believes that the nucleolus is composed of "ergastique" elements of the cell. It is commonly believed that the nucleolus is nothing but reserve food for the chromosomes which may build themselves from this supply. De Mol (1926), Zirkle (1928), and Fikry (1930) suggest that this body may break up into small particles and act as the carriers of hereditary stimuli from the genes through the cytoplasm. Further discussion of these theories, as well as others, will be found in the text.

In addition to a study of the origin and behavior of the nucleolus an attempt has been made in the present study to clarify some of the points concerning the structural behavior of the chromosomes as a group throughout their developmental cycle and to throw light on the question of the supposedly continuous spireme and on the physical relationship often observed between chromosomes and nucleolus. Some of the conclusions are based on observations made from both living and fixed material.

B. MATERIAL AND METHODS

To make a study of this nature as comprehensive as possible, it was thought essential that the observations be made on various plants that are unlike taxonomically. The following plants were used: (a) *Callisia* and *Yucca* (Monocotyledons); (b) *Paeonia* and *Hepatica* (Dicotyledons); (c) *Pinus* (Gymnosperm); (d) *Polystichum* (Pteridophyte); and (e) a species of *Spirogyra* (Thallophyte). Some observations were made from various other plants to see if there could be found any peculiarities not present in those mentioned above. The most intensive observations were made on *Callisia* and *Paeonia*.

It was thought advisable to supplement the study of fixed material with that of living material as an aid in interpreting the phenomena observed. Living material was subjected to various toxic fluids, and

*The use of the terms "sister cells" and "sister nuclei" is suggested when cells or nuclei of the same "generation" are discussed, "daughter cells" and "daughter nuclei" being reserved for cases where derivation is implied. These terms are so used in this paper.

their effects on the protoplasm in general, and nucleolus in particular, were observed, and thus an interpretation of fixed objects could be arrived at.

A fixative of 5% formalin and .5% chromic acid, in equal parts, was the principal killing and fixing solution used. This fixative seems to have been originally developed by Lewitzky (1931) and was recommended by Marshak (1931). The present author has made use of it with success. Its usefulness was proved by applying it to the delicate stigma hairs of *Callisia,* and it was found to cause less disturbance to the cell and its contents than any other reagents commonly used for cytological preparations. With this fixative the nucleolus takes a dark stain both with crystal violet-iodine and iron-alum haematoxylin. In the material fixed with this reagent and stained with crystal violet-iodine both chromosomes and nucleolus retain the stain remarkably well even after long washing in absolute alcohol. For the technical procedure with this fixative the reader is referred to my paper (Dermen, 1932). In addition to this method of preparation the Ehrlich-Biondi differential stain recommended by Van Camp (1924) was used. This is made up of Orange G, acid fuchsin, and methyl green. (For full details the reader is referred to Lee's Vade Mecum, p. 177, 1928). Blotting paper tests of this stain described in this book were found essential and, accordingly, the proportion of reagents could be varied to give the proper reaction.

C. MORPHOLOGY OF CHROMOSOMES IN RELATION TO THE NUCLEOLUS

In order to make clear the nature of the relationship which may exist between chromosomes and nucleolus, it was considered essential that first a thorough analysis of the chromosome morphology should be made. For this reason this phase of the problem was undertaken.

I. Structural Continuity of Chromosomes in Cycle. When the Lewitzky fixative is used, at no period of development are the chromosomes found aggregating into an unrecognizable mass. They always retain their linearity, and at prophase are found within the nuclear membrane as units grouped in the same relative positions which they occupied during anaphase. It seems that the individual chromosomes retain their particular forms (Figs. 48, 49, and 2) as they move to their respective poles during division, bent at their polar constriction points. The prophase chromosomes (Fig. 2) differ from those at anaphase (Fig. 49) only in that the former are more opened up to fill the space inside the nuclear membrane, while the latter are forced together somewhat into a more compact form in moving to the poles. This

compactness is extreme at telophase (Figs. 6, 10, 11, 16, and 17). From this point on, as soon as the new nuclear membrane is formed, there take place reverse morphological changes. A new nuclear membrane encloses the closely grouped telophase chromosomes; then follows the expansion of this membrane, due possibly to expansion of the tightly pressed chromosomes which start to develop into longer and finer threads, to the increase of nuclear sap, and to the development of nucleoli in number and size, resulting in an increase of nuclear volume as a whole. In comparing specifically Figs. 2 and 49 (in Fig. 49 only upper polar group should be considered), prophase and anaphase respectively, one can not fail to be impressed by the similarity of the orientation of the chromosomes. The difference is that in Fig. 2 the chromosomes are longer, spiral and zigzag in appearance, while in Fig. 49 they are short and straight, tending to crowd together as they are forced to the pole, and appear single in structure. It was found, however, after critical study, that even at anaphase the chromosomes possess a dual structure. As in prophase (Fig. 2) and early metaphase (Fig. 29—in this figure the region of primary constriction is illustrated as faithfully as possible), anaphase chromosomes are found split lengthwise and even show twists in the chromatid threads, as illustrated in Figs. 7 and 30, and these twists become more pronounced in telophase, as shown in Fig. 8.

The chromosome contours at anaphase, as illustrated in Figs. 49-51, seem significant, and the interpretation may be that these chromosomes are split and their chromatids twisted together, as shown in Figs. 7, 8, and 30. Similarly, premeiotic chromosomes of microsporocytes show similar contours, indicating that they are already split before microsporocyte chromosomes go through characteristic meiotic development. The split nature of the chromosomes at this stage has been confirmed by a number of reputable cytologists, such as Sharp (1929) and Kaufman (1931), working with plants, and McClung (1928) and Robertson (1931), working with animals. Darlington (1932), however, still maintains that duality of the chromosomes at this stage is due to optical illusion, although previously (1926) he reported seeing chromosomes split at anaphase. This anaphasic split, as maintained by Sharp and others, seems to take place during the late prophase and metaphase. Such a split was seen in the Callisia satellite chromosomes, as illustrated in Fig. 5. The chromosomes at this stage, besides being fully split into distinct halves in readiness for mitotic division, also seem to show in each chromatid a secondary split, part of which, at least, could be distinguished if observed carefully. At the upper end split there were also observed chromomere-like structures in pairs. It may be remarked

here that only rarely can these features be made out clearly. Delicate fixation and staining are primary requisites; and even with the best technique it requires special attention and considerable care on the part of the investigator that this feature may not be interpreted as an optical illusion.

Here may be considered the arrangement of the chromosomes on the metaphase plate in preparation for mitotic division. The metaphase chromosomes (*Callisia*, Fig. 48) have a characteristic orientation which is considered normal; they are arranged in circular fashion, polar (primary) constrictions pointed toward the middle of the plate, and homologous chromosomes tending frequently to show a secondary pairing. If homologues are not always adjacent, at least there seems to be a tendency for them to orient themselves in such a fashion as to facilitate synapsis during meiosis. There may be a strong affinity between homologues at their polar constriction points that will play some part in bringing them together and result in their close pairing at meiosis. Structurally the chromosomes of *Callisia* vary considerably. The total number, as reported by Sax (1932), is 12, of which 4 have approximately median constriction (Figs. 48 and 49), and 8 have subterminal constriction. Two of the latter possess satellites. No secondary constrictions are observed.

It is not uncommon to find in the literature references to a granular stage of nucleus during the resting stage. A careful study of Figs. 18 and 33 perhaps will throw some light on the exact nature of the nucleus at this stage. Fig. 33 represents the telophase of somatic chromosomes from *Paeonia*. The two sister nuclei are viewed from the side and illustrated more or less diagrammatically. At this stage, as was pointed out above, the chromosomes are found already split and showing fine threads, each one representing a chromatid. The appearance of granulation often observed at these stages seems to be due both to curvatures and to points of strongly stained areas (Fig. 18). In mitosis these chromosomes will further elongate and open up to develop finally into a structural make-up similar to that illustrated in Fig. 2. If this stage were viewed from a different angle, it would no doubt present an entirely different aspect. Therefore, if it is true that at prophase the chromosomes hold positions comparable to those at anaphase, then any possibility of their joining end to end into a continuous spireme is inconceivable. This point is further confirmed by the work of Sax and Anderson (1933) and others who show that chromosomes may interlock during meiosis, a situation which would be impossible if the chromosomes were continuous.

The conception of the continuity of the chromosomes in a spireme

has probably been derived from impressions of oblique and polar views of nuclei of stages illustrated in Fig. 1, and especially of early stages similar to those in Fig. 18. (Fig. 1 is of the same stage as Fig. 2, except that in the former the nucleolus is included.) Even in a stage as late as Fig. 1 one can easily be misled concerning the continuity of the chromosomes, but with care they can clearly be made out as separate units. When root-tip sections were crushed under a cover-glass to flatten the cells, it was apparent that in all stages chromosomes invariably show linearity and are never broken up into scattered granules. Martens (1928), having made a similar study from living and fixed material, finds the nucleus is not granular in make-up but primarily reticulate and filamentous, and that there is no continuous spireme at the beginning of prophase; that "Le réseau interphasique n'est donc que l'ensemble des chromosomes télophasiques—à peine plus évolués—et donc les mailles filamenteuses sont reliées par d'autres filaments d'union," and confirms "la persistance—ou mieux, la continuité morphologique et génétique—des structures chromosomiques d'une cinèse à l'autre, au cours de l'interphase et du repos."

II. Chromosome and Nucleolar Relationship. It can be seen from the preceding description of chromosomes that the idea of a continuous spireme is incompatible with the facts, that the chromosomes retain a characteristic arrangement inside the nuclear membrane, and that the individuality of the chromosomes is maintained; therefore a direct flow of nucleolar material, postulated by Van Camp and many others, from the end of a chromosome through the rest of the chromosomes, becomes an impossibility.

Figure 1 shows a large central nucleolus around which the chromosomes are distributed. In this nucleus all the chromosomes are found, with one exception, free from any physical connection with the nucleolus. At the upper side of the nucleolus there was noted the only probable connection at the bend of a chromosome with the nucleolus. I was not able to discover, at this stage, the presence of a pair of satellites characteristic of this species of *Callisia* and determine their relationship with the nucleolus, and only once, after a long search, I found two satellite-like bodies, one on each side of the nucleolus, at a considerable distance from the chromosome ends viewed from the side of the nucleus in a position similar to the one illustrated in Fig. 2; however, these bodies may have been extra-nucleolar bodies which are described below.

Van Camp (1924), using the Ehrlich-Biondi differential stain, found an intermediate coloration on the portion of the chromosomes attached to the nucleolus and therefore concluded that there was a direct flow

of nucleolar material over the chromosomes. Zirkle (1931), by using a selective fixation method, confirms this conclusion and finds that the nucleolar material which has flowed "into" the chromosome threads was fixed at anaphase in a skeleton form. The present author has been unable to confirm the findings of these investigators. In all the cells he has examined there was no sign of intermediate staining between any portion of the chromosomes and nucleolus. The nucleolus at all stages was always bright red and the chromosomes pale blue when material was stained with the Ehrlich-Biondi solution. Fig. 3 is a diagrammatic drawing of a prophase stage from differentially stained material where no such intermediate coloration could be observed. On the other hand, if Zirkle's contention is correct, then the anaphase chromosomes were expected to show some red granular framework. Fig. 6 is such an anaphase stage; the chromosomes were of the same pale blue color as in the prophase in Fig. 3. When *Paeonia, Pinus, Polystichum,* and other species were subjected to similar treatment, the results were the same as those found in *Callisia;* the differentiation was always perfect.

It is quite generally assumed that the nucleolus decreases in volume during the development of the chromosomes, and that it supplies material to the "emerging" chromosomes. The facts on hand, however, seem to indicate that, at least in the plants studied, the situation is not such as described above, and that there is no diminution in size of the nucleolus from the resting stage, when it reaches its maximum volume, until the end of prophase before the nuclear membrane disappears. This fact can be demonstrated clearly by studying meiotic stages of species in which only one nucleolus is generally found, derived, no doubt, from fusion of a number of small nucleoli to form a large one. In *Callisia* there are usually two nucleoli present in the earliest meiotic stage, but as a rule there is only one at later stages. When there are two at early leptotene stage (Fig. 19), they are always smaller than the single nucleoli in adjacent nuclei (Fig. 20). Even at these early stages there are very few cells with double nucleoli, and these unquestionably later result in one nucleolus by fusion. Figs. 21-24 represent the zygotene stage, where the chromosomes are considerably thicker than in Fig. 20 and Fig. 25 the satellite chromosome pair at late diakinesis just before the nuclear membrane disappears, which, in this species, always holds the nucleolus at its satellite end, as reported by Sax (1932). In comparing the sizes of nucleoli in Figs. 20 and 25 and comparing both with the inner nucleolus of Fig. 18, which represents a cell from the earliest stage of meiotic development resulting from the division of the archespores, one is impressed by the constancy in size of the nucleoli all through these developmental stages. Similarly, Fig. 57

shows the diagrammatic representation of the volumes of the two nucleoli from *Hepatica, a* sketched from a very early leptotene, and *b* from the diplotene stage, showing the same constancy in size of the nucleolus as in *Callisia*.

The assumption of transference of nucleolar material to the chromosomes seems to be based on the fact that at later stages the chromosomes take up stain more readily than at earlier stages, a fact which has led many investigators to believe that there is a correlation between the stainability of chromosomes at later stages and entire disappearance of the nucleolus by the time of chromosome division. In *Yucca*, illustrated in Figs. 55 and 56, it is shown that there is hardly any sign of decrease in volume of the nucleolus which is left out in the cytoplasm during meiosis. In general, it appears that the nucleolus reaches a maximum size at a very early stage in the nucleus soon after a nuclear membrane encloses the chromosomes; the size of the nucleolus remains constant all through further development of the nucleus to the very end of prophase, very soon after the nuclear membrane disappears; then the nucleolus generally disappears, the rate depending upon the species of plant, as will be indicated below.

Belar (1928) seems to be justified in stating that there is not sufficient ground for assuming that the nucleolus has any role in the process of building up the chromosomes by increasing their mass. It was shown that when the differential stain was applied at all stages, the chromosomes took a pale blue color, while the nucleolus stained bright red. On the other hand, when crystal violet-iodine, or even iron-alum haematoxylin, is used, the chromosomes throughout do not show the same intensity of coloration as when Ehrlich-Biondi stain is used. Therefore this discrepancy of staining intensity of the chromosomes does not seem to be due to their being less chromatic at earlier stages, and more so at later stages, but rather to their "thinness" or "thickness," depending upon their stage of development. Moreover, the stainability and the degree of retaining of the stain of the fine chromosome threads may be altered with different fixatives and stains.

III. Satellites and Secondary Constrictions. Belar (1928) states that there is no basis for assuming that there is any connection (physiological) between satellites and nucleoli, while, on the other hand, Heitz (1931a and 1931b) has given great importance to these bodies. Heitz's theory is that nucleolar bodies originate on and around the achromatic threads behind the satellites and at secondary constriction areas, and that the number of nucleoli at late telophase is the same as these satellite and constricted chromosomes. De Mol (1927) was perhaps the first to notice a correlation in the number of satellites and

nucleoli. In the present paper is reported a complete study of the satellite and constriction situation in *Callisia, Paeonia,* and *Pinus,* in order to have a basis for analyzing the relationship of the nucleolus to these features.

The somatic chromosomes of *Callisia, Paeonia,* and *Pinus* were subjected to a detailed study to determine the presence and number of satellites and secondary constrictions. Pinus chromosomes were found to be devoid of typical globular satellites, but instead there were present five pairs of chromosomes with secondary constrictions which varied as to their position and length of achromatic threads, though apparently constant for each chromosome so characterized. Fig. 54 illustrates *Pinus Strobus* chromosomes at anaphase (only part of the 12 pairs of chromosomes in each half is shown), showing some of these constricted areas on the chromosomes. *Callisia* (Fig. 48) has a pair of small satellites attached to relatively long threads which were hardly noticeable in this figure but are generally of good size, as shown in Fig. 5. Four plants of *Paeonia* were studied, two of which were of the species *P. suffruticosa* and two of different species,—namely, *P. Delavayi alba* and *P. Woodwardii.* Fig. 50 (*a* and *b*), drawn from two adjacent sections, represents an anaphase from *P. suffruticosa* which was cut into two by the microtome knife. The exact number of the satellites in this species was determined from this, as well as from other cells, and was found to be four, the position of which can be clearly seen. Two satellites are present on the short arms of a pair subterminally constricted, and the other two on the short arms of a submedianly constricted pair. In Fig. 50b in the lower polar group one satellite was distinctly split, indicating a behavior similar to chromosomes at this stage discussed earlier. Here it should be emphasized that the satellites are normal components of some chromosomes and form their characteristic features with permanent attachment and are not free bodies picked up by chromosomes from the surface of the nucleolus, as was suggested by Navashin (1927).

The *Paeonia* species studied had 5 pairs of chromosomes. They varied somewhat in size of chromosomes and satellites and in number of satellites. Fig. 50 represents a young seedling of *P. suffruticosa* from the Arnold Arboretum, while Fig. 51 represents a plant of the same species that has been growing at the side of the Bussey greenhouse. When slides made from these two plants were compared, a constant difference in number of nucleoli was found. The highest number reached in the greenhouse plant (Fig. 51) was always less than that of the Arboretum plant (Fig. 50). On the basis of Heitz's theory it was suspected that this discrepancy in the number of nucleoli in two differ-

ent plants of the same species might be due to a difference in the number of satellites. To test this idea many cells at anaphase and metaphase were crushed by pressing on the cover glass and forcing the chromosomes to spread apart to make their satellited feature more obvious. Strikingly enough, the result was that actually the number of satellites of the plant near the greenhouse was less than in the plant of the Arboretum, three and four respectively. Fig. 51 represents an anaphase stage with three satellite chromosomes at each pole pressed flat and the chromosomes forced to spread. There was one pair of chromosomes submedianly constricted with very small satellites compared with a similar pair referred to in Fig. 50, and only one of the chromosomes with a subterminal constriction possessed a satellite corresponding in size to the ones illustrated in Fig. 50.

Figure 52 is a metaphase stage of *Paeonia Woodwardii* chromosomes. This plate also was crushed and flattened. The division at the polar constriction areas was quite distinct. There were six satellites, two more than in *P. suffruticosa* (Fig. 50), these two additional ones being on the shorter arms of a near-medianly constricted pair of chromosomes, while the other two pairs were on the same type of chromosomes as in *P. suffruticosa*. The satellites on these sub- and near-medianly constricted chromosomes were as large as the satellites of the submedianly constricted chromosomes of Fig. 50, while the ones on the subterminally constricted chromosomes were very minute and close to the end of the chromosomes and were determined only after considerable effort. Fig. 53 is from *P. Delavayi alba*, with one subterminally constricted chromosome being shown which possessed a large satellite quite comparable to the ones in Figs. 50 and 51, in contrast to the small ones in Fig. 52, varying only in the length of the achromatic thread, which was somewhat shorter. In other details the chromosomes of this species were comparable to those of *P. Woodwardii* (Fig. 52). On the basis of polar constriction the chromosomes of all *Paeonia* species reported here may be classified thus: one pair subterminal, one pair submedian, and three pairs (in varying degree) near-median.

D. ORIGIN AND BEHAVIOR OF THE NUCLEOLUS

Considerable space has been given to consideration of two important works by Van Camp (1924) and Heitz (1931a and 1931b) concerning the origin of the nucleolus in plants. Van Camp, applying Ehrlich-Biondi stain, was able to show that the nucleoli originate from the chromosomes at telophase in the form of small globules which later, by fusion, collect into one large nucleolus, while Heitz limits the origin of the nucleoli to the satellite chromosomes, specifically on and around

the achromatic threads that connect either a satellite or a constricted arm with its chromosome. These two findings were subjected to an intensive study and their merits evaluated.

I. The Nucleolus in Somatic Cells. The origin of the nucleolus is best studied in longitudinal root-tip sections of plants with large chromosomes. Fig. 9 represents two sister cells from Callisia root-tip sections. Here are shown, diagrammatically, small globules of nucleolus that were differentially stained red in contrast to the pale blue color of the chromosomes which are not shown. These globules collect, undoubtedly by fusion, into two and finally one body (Figs. 10 and 11) and, as more globules are produced and added together, the nucleolus grows very rapidly and becomes constant in size during further development of the chromosomes. In general, the number of nucleoli in *Callisia* is one or two, and quite rarely three, as illustrated in Fig. 12. All these three nucleoli were homogeneously colored bright red by the Ehrlich-Biondi stain, indicating that they were of the same nature. Whenever it was possible to distinguish the outlines of the chromosomes differentially stained, it was observed that these globules were on the surface region, around and between the split chromosomes. Fig. 31 is from *Paeonia suffruticosa* (greenhouse plant). The granules were found not only where the chromosomes are more compact, but also on the arms of the chromosomes that lay outside the region of the compact mass. Here it can be clearly shown that the origin of nucleolar substance is not associated with the achromatic region of the satellite. Otherwise, assuming that these exposed arms may represent satellite arms, there should have been one granule to an arm, which is not the case. Fig. 32 is a diagrammatic representation of a similar stage, drawn on a larger scale to bring out the approximate number of these globules. Fig. 37 is from *Pinus* and represents the same very high number of small nucleolar globules. In *Polystichum* was found the same situation as in others; however, the higher number of chromosomes in this species ($2n = 60+$) making it difficult to study the satellite or secondary constriction (if present), the representation of this situation was limited only to plants in which the number of satellites and secondary constrictions was determined so as to have a sound basis for discussing satellite and nucleolar relationship. Because of the same difficulty, a species of *Arisaema* (*A. triphyllum*, collected near Pepperell, Mass.) with $2n = 60+$ chromosomes is not illustrated, but this also showed the same numerous small globular nucleoli at a stage similar to that illustrated for *Paeonia* and *Pinus* in Figs. 32 and 37 respectively.

In Fig. 29 is shown a part of a satellite chromosome from an early metaphase plate of *Paeonia suffruticosa* (Arboretum plant) to illustrate the supposedly true relationship between a satellite and the nucleolus. This small nucleolus is, perhaps, a remnant of a larger nucleolus that has not completely disappeared in the cytoplasm. It can be seen not only that this nucleolar body does not surround the achromatic thread—contrary to Heitz's theory—but also that it lies away from the thread and is found very near the satellite (there may actually be an attachment between them). Both McClintock (1931) and Burnham (1932) have shown very clearly in *Zea Mays* that the nucleolus lies attached at the side of the achromatic thread of the satellite and not around it. In *Callisia* (Fig. 22, meiotic stage) and *Paeonia* (Fig. 29) this association seems to be between the satellite and nucleolus instead of between the achromatic thread and nucleolus.

A behavior of extrusion of the nucleolus into the cytoplasm in microsporogenous cells will be more fully described below. In Pinus somatic cells not a trace of the nucleolus was found at metaphase, as was also reported by Zirkle (1931); in *Paeonia* very rarely one or two small pieces were found just before mitosis (Fig. 29); in *Callisia* often there were found one or two pieces just before (Fig. 4) and after (Figs. 16 and 17) mitosis. A similar phenomenon was observed in Polystichum root-tip sections. Fig. 45 is a diagrammatic representation of the metaphase from side view. Outside the division sphere there is a nucleolar body that is a remnant from an earlier division stage. Similarly, in Fig. 46, is shown a large nucleolus outside the nucleus which is in prophase stage. Fig. 47 shows a nucleus with a typical nucleolus from an active region. The large nucleolus in the nucleus shows a bag-like feature as if containing a number of free bodies inside a bag, while the ones outside in the cytoplasm (Figs. 45 and 46) are uniformly round and homogeneous. Besides the large nucleolus shown in Fig. 46 there are a number of small nucleolar particles, staining red like the large ones, which may aggregate into various forms, as shown in Fig. 45, and finally be extruded into the cytoplasm, where they may further coalesce into a more compact form (Figs. 45 and 46). This peculiar form of nucleoli in such small granules may be comparable to the so-called amphi-nucleoli described by Wilson (1925). No speculations are justified here as to either their analogy or homology, since the present author has not made a study of this situation in animals. However, in *Polystichum* these particles show the same behavior as is characteristic of larger nucleolar bodies, indicating that they are of the same nature as the large ones. Extrusion of nucleolar material, in part or in whole, is reported by many investigators, among whom may be mentioned

Davis (1903) and Wakayama (1930), working with fungi, Yamaha and Sinoto (1925) on some thirty species and forms of phanerogamic plants, while in some Protista (Belar, 1928) the nucleolus does not disappear at all during mitosis but divides into two which are included in the sister nuclei.

In fixed material of *Callisia,* as well as in living, in the stigma hair nucleus there often were found around the region of the nucleolus some small extranucleolar bodies (Fig. 13). When subjected to differential stain, these bodies differed in their coloring when compared with the bright red of the nucleolus,.their color being of a darker shade of red. According to Heitz (1928), these bodies are considered to be chromosome fragments, but recently this assumption was refuted by Scheuber (1932), who finds that these bodies vary in number, size, and shape and show differences in staining when compared with the nucleolus. In *Callisia* the situation was found to be identical with that reported by Scheuber. When fixed material is studied carefully, one will generally find a clear area around the nucleolus, no matter how small it may be, while around these bodies no such clear area is observable, and their staining is intermediate, though more like nucleolus than chromosomes.

Similar bodies were observed in the microspore cells of *Callisia.* Fig. 27 represents a late prophase stage with six chromosomes, a large nucleolus in the middle, and a body at the side. This body can not be confused with a satellite, since the four short chromosomes point away from it and also because of its characteristic stainability referred to before. Fig. 28 is the drawing of a metaphase with such a body at the distal end of the satellite chromosome. Again it should be stated here that this body stained characteristically and was no doubt an extranucleolar body. It seems to have a remarkable stability in size and does not disappear as the nucleolus would. Identical bodies were observed during anaphase and telophase where it could be seen that they are left outside the new nucleus in the cytoplasm, there perhaps to disintegrate eventually. Such bodies were also found in root-tip cells of *Callisia.*

II. The Nucleolus in Microsporogenous Cells. Undoubtedly the process of nucleolar origin in the telophase of archespores is the same as was described for the somatic cells of root-tip material. No favorable stage having been found, this point could not be illustrated.

Figures 14-17 are from very young microsporogenous tissue of *Callisia.* At this stage quite commonly were found large extranuclear nucleolar bodies. In Fig. 15 there are two sister cells that have developed at a parallel rate, both being at late prophase stage. At the outside of each nucleus there is a large nucleolar body in an opposite

position to the corresponding one in the sister cell. Undoubtedly these two extranuclear nucleoli are remnants from a like cell shown in Fig. 14. Figs. 16 and 17 show the behavior of a nucleolus that has not been dissolved completely during the earlier stages of mitosis and is forced into the cytoplasm where it may remain for a considerable time during later stages of meiotic development, since these cells are destined to become microsporocytes. Fig. 18 represents the earliest stage of a microsporocyte after emerging from a mitotic division similar to the one shown in Figs. 16 and 17. In this, besides a large nucleolus being present among chromatic threads, one medium-sized nucleolus is found outside the nucleus in the cytoplasm probably resulting from a process illustrated in Figs. 15-17. In Fig. 23, which represents a much later stage, a nucleolus is found outside very near to the main nucleolus inside the nuclear membrane to which two chromosomes seem to be attached, while there is still another extra round body within the nucleus which is free from any such connection. Inside the cell represented in Fig. 21 there are present two such free round bodies, besides the larger nucleolus with a chromosome attached to it, that also show no connection with any chromosomes. In Yucca microspore mother cells there are either two medium-sized or one large nucleolus present inside the nucleus, as shown in Fig. 55. This figure is drawn at the nucleolus level, and not all the chromosomes are included in it. It must be emphasized here that the nucleolus is free from any chromosome attachment. The nucleolus in *Yucca* retains its size till the time of disappearance of the nuclear membrane, then it is extruded away from the division plane, sometimes one nucleolus to one side and another to the other side of the division plane, or both to one side individually, or two closely together, as shown in Fig. 56, there perhaps to disintegrate gradually. The nucleolar extrusion referred to in *Callisia* and *Yucca* may be analogous to a similar but seemingly more common behavior in *Polystichum*.

As is described above, the nucleoli that have not completely dissolved after the disappearance of the nuclear membrane are not retained in the newly-forming cells but are forced outside; so this behavior may be considered the primary rule but perhaps not the only rule. As is the case in some Protista described by Belar (1928), Christoff and Gentscheff (1932) have assumed that the nucleolus divides during mitosis, and the pieces of these nucleoli are retained in the newly-forming sister nuclei. So far most of the findings by many investigators and the present author indicate the contrary. However, in the present study there was encountered a difficulty which may be explained if it is assumed that rarely some of these pieces of nucleoli may be enclosed among the dividing chromosomes and retained in some of the nuclei.

As was pointed out, the nucleolar pieces shown outside the nucleus in Figs. 15 and 18 took a bright red color similar to the ones inside, while the free nucleoli inside the nucleus in Fig. 21, one outside and one inside the nucleus of Fig. 23, stained somewhat differently. If these free bodies are actually carried over from an earlier cell, the age of these nucleolus-like bodies may account for their reaction toward the stain, thus staining somewhat differently than the true nucleolus. They may remain there as inert particles. The above is presented as a mere suggestion, since the presence of these extra bodies is difficult to explain in the absence of experimental data. These bodies in the microsporocytes are of rare occurrence.

Another feature in *Callisia* was the presence of a small bud-like protuberance on the nucleolus, as shown in Fig. 22. A phenomenon similar to this is reported by Gates and Latter (1927), Maeda (1930), and Selim (1930). Gates and Latter find at the points of chromosome attachment to the nucleolus dark staining bodies, varying in number, size, and distribution. Maeda similarly finds small nucleolar bodies on the "mother nucleolus" and sometimes free from it in the nuclear cavity. Selim, studying meiosis in rice, finds the nucleolus budding off a "secondary nucleolus" at late diakinesis. He supports the view that the secondary nucleolus contributes material to the chromosomes, while the primary may contribute to the spindle.

Similarly, as in mitotic sister nuclei, in the sister nuclei of first meiotic division there were observed a number of small nucleolar globules (Fig. 26) which were found clearly oriented along the chromosome threads and not in the clear space of the nucleoplasm. Owing to some technical difficulties arising from poor fixation, no critical drawings of meiotic stages of *Paeonia* and *Pinus* could be presented. Only in an outline fashion the number and depth of nucleoli in *Pinus* (*P. Thunbergii*) from earliest to latest stages of meiosis are given (Figs. 39-44) to show the chromosome and nucleolus relationship. However, no radical differences from *Callisia* were observed in these plants. In *Paeonia suffruticosa* (greenhouse plant—other members of this genus were too young for the study of the meiotic phase of the problem) the nucleoli in the earliest stage numbered from six to one, while at later stages the number decreased to three to one, disappearing with the disappearance of the nuclear membrane. In *Pinus* at early leptotene (Fig. 39) and during early diplotene (Fig. 40), as many as nine nucleoli were found, the number decreasing as the development of the nucleus advances, but the volume of total nucleoli not showing any decrease. Fig. 41 is from late diplotene, while Fig. 42 is from late diakinesis stage when the nuclear membrane is disappearing or newly disappeared prior

to the chromosome arrangement in a metaphase plate. Fig. 43 is one of the sister cells after the first reduction division, while Fig. 44 is after homotypic division before the nuclei go into resting stage. In these two figures the number of nucleoli reached as high as nine to twelve.

III. RELATIONSHIP OF NUCLEOLUS TO SATELLITES AND SECONDARY CONSTRICTIONS. Heitz (1931a and 1931b) has made an extensive study of satellite and secondary constriction number in *Vicia* and other plants upon which he has based a definite theory concerning the origin of the nucleolus. His conclusion is that the number of nucleoli in any species in telophase sister nuclei should correspond to the satellites and secondary constrictions there present, for he assumes and gives evidence, as was mentioned above, that each nucleolus at telophase originates on and around the achromatic thread of each satellite or secondary constriction in the form of a collar. This correlation could be more readily proved if there were never found more nucleoli than satellites and secondary constrictions characteristic of a species. However, it is significant that the number of nucleoli in the greenhouse plant of *Paeonia suffruticosa* never reached the highest number found in the Arboretum plant of the same species. In the former the highest number was found to be five (Fig. 34), while in the latter seven, which have respectively three and four satellites. Both *P. Woodwardii* and *P. Delavayi alba* have six satellites; in the former the number of nucleoli reached as high as nine (Fig. 35), while in the latter quite rarely to as high as eleven (Fig. 36). Similarly, in *Callisia* quite rarely there were found three nucleoli (Fig. 12) instead of two to correspond with the number of satellites; in *Pinus* (*P. Strobus* root-tip) the number reached as high as fourteen (Fig. 38) where there should have been only ten if there are ten achromatic areas in the twelve pairs of chromosomes. There can be no doubt that a discrepancy to Heitz's expectation exists, the cause of which should be looked for elsewhere. However, the above data clearly indicate that whenever there is an increase of satellites or secondary constrictions, there is a similar increase in the number of nucleoli. The analysis of the situation is given below.

IV. NUCLEOLAR SYMMETRY IN SISTER NUCLEI. Before I had occasion to review the literature on the nucleolus, the perfect symmetry in some sister nuclei in root-tip sections, as illustrated in Fig. 33, had impressed me, and I was led to assume that this symmetry was due to the spatial relationship existing between the chromosomes in the sister nuclei. Therefore the mirror image of some nuclei in having nucleoli similar in number, size, and position was thought to be primarily due to the proportionality of the spaces between the chromosomes of the

sister nuclei; hence the symmetry of nucleoli in these cells. However, further work on this phase made it necessary to alter somewhat this original assumption.

Fig. 33 is a somewhat diagrammatic representation of the symmetry between four nucleoli in two sister nuclei. The symmetry is not only in number but also in size and position of the nucleoli. This drawing is from a root-tip section of *Paeonia suffruticosa* with the four satellites (as illustrated in Fig. 50). According to Heitz, the size of a nucleolus depends on the length of the satellite achromatic thread and its position on that of the satellite. In Fig. 33 the four nucleoli show a symmetry of number, size, and position of nucleoli but not a symmetry of the type expected according to Heitz, since in this plant two satellites are proximal and two are distal to the division poles (Fig. 50), while here one nucleolus is proximal and three are distal. There is also a size difference; the polar nucleolus in each nucleus seems to be more than the total volume of the other three nucleoli. Heitz would explain this on the basis that the increase of a nucleolus is proportional to the space around it; however, there were found many cases, as illustrated in Fig. 34, where there are small nucleoli which seem to have ample space to draw material and grow in size if Heitz's assumption is correct, but nevertheless they have remained small.

De Semet (1913) has proposed a genetic relationship between certain chromosomes and the nucleoli on the theory that nucleoli originate from certain chromosomes, and owing to this relationship he assumed the existence of a symmetrical relationship between sister nuclei. Yeates (1925) and Sprumont (1928) have supported de Semet's view, while Abele (1930) has explained this symmetry by assuming that "Die Nukleolar-substanz wird bei der Karyokinese in gleichen Mengen auf beide Tochterzellen verteilt, infolgedessen sind die Nukleolen oder Nukleolensatz beider Tochterkerne gleich gross."

It was pointed out earlier that the number of nucleoli during the early stages of nuclear development corresponded quite closely to the number of satellites present for the species in question, although quite often there were found more nucleoli than were expected according to Heitz's theory. However, since there seems to be some correlation between the number of satellites and the number of nucleoli, and also since during meiosis the nucleolus is constantly attached to a satellite chromosome pair in *Callisia* (Figs. 21-25), (Sax, 1932), in *Ranunculus* (Sorokin, 1929), and in *Zea Mays* (McClintock, 1931 and Burnham, 1932), therefore the symmetry often found between two sister nuclei in the number, size, and position of nucleoli is suggested to be primarily due to a physical relationship between a satellite and a nucleolus, and

independently to corresponding spaces between the same set of chromosomes in the sister nuclei, where nucleolar globules "exuded" from the adjacent chromosome surfaces fill in. The decrease in number and increase in size of the nucleoli may come about from the increase in these interchromosomal spaces during the thinning of the chromosome threads and increase in nuclear volume as a whole, allowing these spaces to fuse into fewer and larger spaces, followed closely by the fusion of smaller nucleoli into fewer and larger ones.

V. The Number and Size of Nucleoli in Polyploid Races. De Mol (1926 and 1928) has made some assumptions concerning the size and number of the nucleoli in the di-, tri- and tetraploid varieties of Hyacinths and has concluded that the size of "complex nucleoli" and the number of "simple nucleoli" are proportional to the number of the chromosomes. He believes that after nucleolar globules have been formed during telophase, they fuse into a "complex nucleolus" which later fragments into two, three, or four "simple nucleoli," depending upon whether the variety is diploid, triploid, or tetraploid. The present author has observed the process of fusion between two nucleoli at prophase in living tissue but has never observed fragmentation. If fragmentation ever occurs, certainly it must be in very rare circumstances instead of being the rule. The abundance of higher number of nucleoli in early stages of nuclear development and lower number in later stages must mean one thing only, that this decrease of number and increase in volume of nucleoli come about through fusion of higher number and small sizes into lower number and larger sizes. Here may be reported the results of my own observations from diploid, triploid, tetraploid, and pentaploid *Petunia*.

The largest single nucleoli from longitudinal sections of root-tips of six plants (Table 1) were measured in order to establish the size relationship of nucleoli in the polyploid series. The first two of these plants were diploids (7Lx and $7Lnc_{2-8}$), the third a triploid $(14S_{2-28} \times 7Lnc_{9})$-1, the fourth and fifth tetraploids ($14S_{2-29}$ and $14L_{2-21}$) and the last a pentaploid (Trip.4 $\times$ $14S_{2-29}$). For the description of these plants the reader is referred to the author's paper on "Polyploidy in Petunia," 1931. On the slide of some of these plants there were either two or three root-tips in serial sections; in that case ten measurements were taken from each root, one from each serial section. It must be remembered that on one section there are hundreds of cells and as many or more nucleoli; therefore, even if only one measurement is taken from a section, it is from one of the largest among hundreds of nucleoli. During the measurements it was found that there was

some constancy in the size of the largest nucleolus for all the sections of the same root; therefore, taking measurements from more sections of the same root-tip was not considered essential. Further, in order to make these measurements more proportionate and uniform, only the largest and circular appearing nucleoli were chosen to avoid confusion and error in the measurements. Below, in Table 1, are given the diametric measurements of nucleoli in microns.

It is apparent from Table 1 that no significant conclusion can be arrived at as to the increase in volume of nucleoli from the increase of chromosomes of diploid to polyploid series, perhaps for the following reasons: (1) the number of measurements appears to be small and (2) the races tabulated here are of mixed nature. At present, unfortunately, there are no available plants to make this study more extensive; therefore, of necessity, the above measurements had to be limited to the numbers presented in this table. Even though these numbers are perhaps small for an exact conclusion, it seems evident that in order to have a basis of comparison on this phase of the problem, one is required to choose plants of polyploid series that are of the same origin. Table 1 shows that even though the second diploid plant (7Lnc) is from a mutated bud from the first diploid (7L), the difference in their measurements is quite striking. The 4n plants and the 5n are derivatives from an entirely different race of diploid, while the 3n strain is from a cross of this 4n and the second 2n race. Perhaps in the case of 3n the small measurement in general may be explained by assuming that the second 2n plant had a diminution factor affecting at least the size of the nucleolus. The two tetraploids and the pentaploid measured practically the same and were somewhat smaller than the first diploid.

In the writer's paper on *Petunia* is discussed the origin of these polyploid strains. From an ordinary diploid race the writer obtained a tetraploid plant, and from these two strains were obtained some triploid strains, and finally a pentaploid strain was obtained by crossing a triploid with a tetraploid. From the old slides of these strains, cross sections, similar measurements of nucleoli were taken. The origin of all polyploid strains, as can be seen, is from a pure-breeding diploid plant; therefore, the measurements from these plants are expected to give more of the true picture concerning the differences in nucleolar volume (Table 2). In this case measurements were taken from ten serial sections of one root-tip from each plant.

Table 2 seems to indicate an increase in diameter of a half a micron from 2n to 3n, one micron from 3n to 4n, and a decrease of one micron from 4n to 5n. There seems to be no difference between the 3n and 5n strains. From these measurements, as from those in Table 1, it may be

Table 1

Petunia series			Nucleoli measurements in microns of ten sections from each root-tip																Range	Median
Strain	Pedigree	Root-tips	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0		
2n	7L⊗	1st								2	8								5.4–7.0	6.2
		2d														3	2	5		
		3d											4	1	1	2	1	1		
2n	$7Lnc_{2-8}$	1st			1	1	1	4	2	1									4.0–5.4	4.7
		2d				5	2	1		2										
		3d	1	6	3															
3n	$(14S_{2-28} \times 7Lnc9)$-1	1st		2	2	4			1		1								4.2–5.8	5.0
		2d			1	1	1	1			4	2								
		3d			1	3	1	1		4										
4n	$14S_{2-29}$	1st									2	7	1						5.4–6	5.7
		2d								1	1	6	2							
4n	$14L_{2-21}$	1st										2	3	3	2				5.2–6.4	5.8
		2d							1		1	8								
5n	Trip.4 xTet.$_{2-29}$	1st											2	6	1		1		5.4–6.8	6.1
		2d								4	5	1								
		3d									2	4	2	2						

difficult to conclude whether or not there is any increase or decrease in the volume of the nucleolus along with the increase of chromosome number from diploid to polyploid series, according to de Mol's theory. However, there may be a tendency toward slight increase in nucleolar volume, due to an increase in number of chromosomes, and also some other factors may be involved in influencing the volume, as in the case of the difference between the two diploid strains in Table 1 and the equality of volume in the triploid and pentaploid in Table 2.

Table II

Petunia strain	Nucleoli measurements in microns of ten sections of one root-tip from each strain														Range	Median
	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8		
2n	1	2	4	3											5.2–5.8	5.5
3n				2	3	2	3								5.8–6.4	6.1
4n						1	3		1	1			2	2	6.2–7.8	7.0
5n				4	4	2									5.8–6.2	6.0

E. THE BEHAVIOR OF THE NUCLEOLUS IN LIVING TISSUE

An intensive observation was made on Callisia stigma hairs, kept alive either in tap water or 5% sucrose solution, in order to have a basis for determining how the fixed material differed from, or how closely it resembled, the living with regard to the behavior of the nucleolus and its relationship with the chromosomes. Entire pistils or styles with stigma hairs were placed in a drop of water on a slide and covered with a cover glass, and the material was studied under low or high power magnification with oil-immersion objective. These hairs, a few scores in number to a stigma, are fine and non-septate cells reaching a length of three millimeters, and were found to be very favorable material.

I. The Area around the Nucleolus. The nuclei and nucleoli in the hairs are considerably larger than those in some other parts of the somatic tissue, such as the style cells, where they may be one-fifth as large. The nucleoli in these cells may measure 4 to 10 microns in diameter, small in the very young and large in the hairs just before the bud opens. With a slow change of form of the nucleus, the nucleolus also changes its position and shape, perhaps owing to pressure exerted by the chromosome threads of the ever moving nucleus in the streaming cytoplasm. There was never found a clear area (separation area) between the nuclear reticulum and the nucleolus. This area appeared whenever these hairs were subjected to a disturbance by introducing a

toxic fluid under the cover glass, resembling the area so commonly observed around the nucleolus, inside the nucleus, of fixed material. Based on this observation the conclusion may be drawn that the clear area around the nucleolus in the fixed material is due to fixation and therefore is an artifact. Fig. 13 represents, in an outline, a nucleus in which is shown the clear area around a vacuolated nucleolus. As a result of any slight injury, physical or chemical, the nucleolus, as if sensitized, immediately pulls itself to one side, somewhat shrunken, close to a globular extranucleolar body (if any is present). Simultaneously, similar shrinkage takes place in the nucleus, enlarging this clear area. The creation of this area seems to be primarily due to sensitizing of the protoplasm (used in a broad term) and secondly, if at all, to plasmolysis. This assumption is substantiated by the fact that, in some cases, when the hairs were treated with Lewitzky's fixative, a clear area was created immediately but very soon disappeared, and no appreciable area was left between the nucleolus and nuclear mass, while when a stronger reagent was used, such as Bouin's or Flemming's, the effect was permanent.

II. EXTRANUCLEOLAR BODIES. In the earlier part of this paper reference was made to the nature of the extranucleolar bodies in somatic cells in connection with their difference in stainability and their possible homology with extranucleolar bodies in meiotic cells. In the stigma hairs of *Callisia*, as illustrated in Fig. 13, these bodies are generally found close to the nucleolus. Whenever the hairs were subjected to toxic fluids, the nucleolus seemed always to pull itself away from the nuclear mass and to remain attached to one of these bodies like a balloon attached to a mast. No adequate explanation can be given at present as to the origin of these bodies, small in size and varying in number from one to five or more. Because of their being near the nucleolar region, they may be considered some sort of extruded material from the nucleolus, or perhaps pieces of nucleolar material that were carried and held among dividing chromosomes and were included inside the nucleus and retained there along with the nucleolus as inert pieces of nucleoli. The present author is inclined to consider the last assumption more likely than the first. In general there was only one body of this kind present in the nucleus, and often there were nuclei without it. It was said that they were always near the nucleolus, but never were any of these found fusing with the nucleolus, which may be considered indicative of their difference, although the nature of this difference is not known.

III. CHROMOSOME AND NUCLEOLAR RELATIONSHIP. One of the principal reasons for studying living tissues was to determine what rela-

tionship exists between the chromosomes and nucleolus. In the style where cells could be found in late prophase stages, the association between the chromosomes and nucleolus was identical with that illustrated in Fig. 1. Obviously the chromosomes are separate units, and many at least are free from any connection with the nucleolus. In the stigma hairs the nucleus appeared very finely granular and compact but was undoubtedly made up of fine threads which never thicken, perhaps because of the lack of cell division in these hairs. The hairs themselves grow in length as the pistil matures and dry up and die after they have played their part (if they have any) during anthesis. With the growth of the hairs there is an increase both in nucleus and nucleolus and a slight decrease (at least in nucleolus) when the hairs become exposed at anthesis.[1] These hairs were treated with fixative to bring about a clear area around the nucleolus to see if there was any close connection between these fine threads and the nucleolus. The only apparent connection which could be found was that between the extranucleolar body and the nucleolus; and sometimes, in addition to some achromatic threads, there were very rarely found threads crossing this clear area, apparently being dragged by the nucleolus, owing to the separation of the nuclear mass and the nucleolus. Therefore it seems that even at early stages there is no complete direct association between all the chromosomes and the nucleolus and that a single attachment point between a chromosome and a nucleolus can not be considered of any physiological importance.

IV. The Nucleolar Vacuoles. Contrary to Fikry's belief (1930) that vacuoles in the nucleoli are artifacts, the present author found that vacuolation was one of the most important features observed in living as well as in fixed material. At times the nucleoli in all the stigma hairs were found to be vacuolated; at other times only part of them were vacuolated; and at still other times there were stigmas with hairs none of which contained vacuolated nucleoli. It is apparent that vacuoles are not permanent features (in the strict sense of the word) of the nucleoli, but vacuolation may be considered a normal phenomenon, and

[1]As a record I should like to mention that in the case of *Callisia* the size of the nucleolus is not constant all through the plant system. In microsporogenous tissue it is constant in all cells, at all stages, measuring about 4 microns; in the stigma hairs it varies from 4 to 10 microns, depending upon the stage of growth of these hairs; in the tissue of style and stamen filaments it measures about 2 microns; while in root-tips, in regions where there is active division, the size may be about 4 microns, and in the epidermal and root-cap cells about 2 microns. In all these cases there is a correlation between size of nucleolus and nucleus. The differences here recorded may be due to difference of nutrition, so that whenever the supply of food is less, there is a corresponding diminution in the size of both nucleus and nucleolus and vice versa.

vacuoles may appear and disappear normally. To check up this assumption, stigma hairs in water cultures were kept as long as they could be kept alive to see if non-vacuolated nucleoli would become vacuolated.

For this experiment young pistils or styles with stigma hairs cut off from pistils were put in a drop of water on a slide and covered with a cover glass. They were first studied under the microscope with oil-immersion objective, the desired regions spotted, the hairs and nucleolus in its surroundings sketched, and the nucleolus measured if necessary; then the slides were put away in a petri dish. At the bottom of the petri dish was placed some wet filter paper to create a moist chamber. This method enabled me to keep some of this material alive in tap water for six days. After many trials not a single unvacuolated nucleolus had become vacuolated. Some appeared to contain vacuoles in the form of small droplets, but this proved to be a sign of degeneration which was followed by the death of the cell.

The general effect of this treatment was that in the living cells the nucleoli always showed considerable decrease in volume. One nucleolus was measured soon after the material was put on the slide. It measured 9 microns in diameter; at the end of the second day it had decreased to 6 microns, to 4 microns at the end of the third day, and to 1.8 microns at the end of the sixth day; and finally the cell died. Results of this kind would lead one to suppose that the nucleolus was used up as reserve food by the "starved" cell and hence its extreme decrease in size. However, a similar decrease was noticed in the nucleus itself but not in the same proportion (no measurements of nuclei were taken).

Analogous behavior is reported by Meyer (Guillermond et al., pp. 183-184, 1933), who believes that the nucleolus is made up of "ergastique" elements of the cell and that the volume of the nucleoli is essentially variable during the physiological state of the cell. The volume of the nucleolus increases when the cell has an abundant supply of nutritive material and diminishes when the cell is in a starving condition. In the mesophyll of *Galtonia candicans* leaves Meyer finds that there is a diminution in the volume of the nucleolus when these leaves are etiolated and states: "Si, dans la feuille vivant dans des conditions normales, on exprime ce volume par l'unite, on constate qu'il s'est abaissé à 0,38 dans une feuille maintenue à l'obscurité pendant 36 jours et à 0,18 dans une feuille maintenue à l'obscurité pendant deux mois." During the change of the albumen of *Galtonia* this author finds some interesting changes in nucleolar volume which are described thus: "dans les cellules de l'albumen jeune, les nucléoles mesurent 52,3 environ; leur volume s'accroît à mesure que l'albumen se charge de matériaux

nourriciers, jusqu'au volume de 101,3; puis il diminue au moment de la formation des parois cellulaires, et surtout au moment de la germination et de l'utilisation de réserves, jusqu'à devenir insignifiant."

To supply food matter for the cells to prevent them from "starving," some material was kept in 5% sucrose solution in vials and on slides. In this solution it was possible to keep these hairs alive for eighteen days. At the end of this time, owing to the growth of yeast cells and molds of various kinds, the tissue as a whole was destroyed. The striking difference between water and sugar cultures was that while there was invariably a decrease of nucleolus in the water, there was hardly any appreciable decrease in the sugar solution, except that whenever the water under the cover glass had evaporated in part, the nucleoli were found rounded up and the flow of the cytoplasm slowed down. As soon as more water was added again, the cytoplasm increased its speed of flow, and the nucleoli took on ellipsoid or other shapes due probably to the activation of the nucleus. In one case one cell was recorded dead because there could not be observed any movement in the cytoplasm, but when water was added, it soon revived and showed cytoplasmic streaming.

It was observed that the vacuolated nucleoli did not all lose the vacuole when they were kept in sucrose solution, except in a few cells, indicating that the tissue can live normally in the sucrose solution, for a while at least, while if kept in tap water, some radical change (superficially physical) seems to take place. During these experiments, however few in number, no vacuolation, *de novo*, was observed even in the sugar solution. This was perhaps due to the somewhat abnormal condition in the sugar solution (if it is assumed that vacuolation is a normal phenomenon), since this solution can not be considered an ideal medium for tissue culture.

Some measurements were taken of nucleoli, some of which were vacuolated and some not. In all cases there was a decrease in volume in water cultures irrespective of the vacuole. Exact measurements were taken of two nucleoli, one with a vacuole and the other without. For convenience, let the vacuolated be No. 1, and the nonvacuolated No. 2. The diameter of No. 1 was 6.2 microns and that of its vacuole 5 microns. The diameter of No. 2 was also 6.2 microns. Overnight, sixteen and a half hours, the vacuole of No. 1 had disappeared, and the nucleolus had decreased in size to a diameter of 5.4 microns. The diameter of No. 2 also had decreased to 5.6 microns. The measurements after 26 hours were, for No. 1, 4 microns, and for No. 2, 4.2 microns. After some hours both cells were dead. As can be seen from these measurements, the disappearance of a vacuole does not seem to have any appre-

ciable effect on the volume of the nucleolus. Therefore, it is perhaps safe to assume that the vacuolated region has been transformed into true nucleolar substance by some physiological phenomenon, the exact nature of which it is difficult to ascertain. This region appears to be of the same concentration as the cell sap. This assumption is based on the fact that in one case a small proplastid-like body was found inside this region and showed the same rate of Brownian movement as took place outside the nucleus.

F. GENERAL DISCUSSION AND CONCLUSIONS

I. CHROMOSOME MORPHOLOGY AND CHROMOSOME-NUCLEOLAR RELATIONSHIP. Conclusive evidence is presented in the text to show that the chromosomes in somatic nuclei keep a form of linearity which remains practically constant from anaphase to the end of prophase; that there exists, in general, a permanence in position and structural form during these same stages. The facts seem to indicate that at no time of development do the chromosomes pass a period during which time they are in a granular state and thrown about in a haphazard way, later to reestablish themselves in threads, joining end to end, again to break apart into separate units as chromosomes.

There is no doubt, among those familiar with karyokinetic problems, of the individuality of chromosomes in structure. The truth of this matter seems to have been substantiated by Rable as early as 1885 and by Boveri (1909) and, more recently, by Kagawa (1926), Belar (1929), Koerperich (1930), and others. Rau (1930) states that in *Cyanotis cristata* pollen-mother-cells no continuous spireme appears to be formed at any stage. It is a fact that generally, morphologically, the chromosomes of a basic set in a species differ considerably from each other; therefore the possibility of diverse chromosomes, such as described for *Callisia* and *Paeonia,* forming a continual spireme is inconceivable. The conclusion may be that, normally, the chromosomes never join end to end. This point may be further emphasized by referring to the work of Sax and Anderson (1933) and others who show that during meiosis some chromosomes may interlock, a phenomenon which could not take place if these chromosomes were parts of the same continuous spireme thread. These facts indicate that the direct flow of nucleolar material into and through all the chromosomes, as suggested by some investigators, is an impossibility.

II. ORIGIN AND DEVELOPMENT OF THE NUCLEOLUS. It is shown that the nucleolus originates in the form of small globules on the surface of the chromosome threads during the late telophase stage, that

very soon there is a close grouping of chromosomes, and that a demarcation between the chromosomes as a group and the outside cell-sap and cytoplasm is established. It is difficult to show if there is a definite nuclear membrane, as such, around the chromosome group at this period, but for practical purposes it may be assumed that such a membrane exists from the beginning of the late telophase till the time of metaphase plate formation. The method of origin of the nucleolus here reported was first described, I believe, by Van Camp (1924).

The process of nucleolar development appears to be a surface phenomenon which can be explained by assuming that a chemical reaction takes place between the substance on the surface region of the chromosomes and the nuclear sap. The nucleolus, then, may be considered a by-product of the chromosome matrix and nuclear sap and, in this sense, may be identified with the matrix, as was suggested by Marshak (1931), but with this difference, that it is the matrix which produces the nucleolar substance by going into a chemical combination with some substance in the nuclear sap. The difference is indicated by their reaction to the differential stain. From this it may be deduced that there exists a striking difference between the nuclear sap inside a nuclear membrane and the cell-sap outside this membrane, because of the fact that the nucleolus will originate and develop inside a nuclear membrane and float in the nuclear sap, while outside this membrane the nucleolus disappears and apparently is dissolved in the cell-sap. It appears, therefore, that the cell-sap has a dissolving effect on the nucleolus, the rapidity of which may differ in different species; hence the lagging of nucleolar particles in some species and the lack of it in others which may be assumed to depend on the varying strength of reaction of cell-sap in different species.

The phenomenon of lagging may also be explained on the basis that there exists a difference in rate of chromosome development during the late prophase and metaphase, varying in different species. For instance, it was noticed that the chromosomes in *Callisia* (somatic stage) are well developed by the time they are forced onto the metaphase plate, already well split, and ready to divide and move toward the division poles. In *Paeonia* and, to a lesser extent in *Pinus*, this development is not so far advanced as in *Callisia* before the nuclear membrane disappears; the chromosomes of the former species stay at metaphase considerably longer, while in the latter the separation of sister chromosomes is much more rapid. It is suggested that into the phenomenon of lagging of nucleolar particles the time element may enter. Thus, in species where the chromosomes divide more rapidly at metaphase, as in *Callisia*, more nucleolar particles are observed during the metaphase

and anaphase; but rarely in *Paeonia* and never in *Pinus*, where the division of chromosomes is delayed, are these particles found.

Heitz recently has developed a theory to explain the origin of the nucleolus based on the fact that in sister nuclei there often exists a symmetry in nucleoli number, position, form, and size. He believes that there is a correlation between number and position of satellites and secondary constrictions and number and position of nucleoli in somatic cells, and that the nucleolus originates in the form of a collar around the achromatic thread that holds the satellite or secondary constriction segment to the end of the main chromosome body. Brunn (1932) indicates that *Primula seclusa* does not possess satellite chromosomes but possesses nucleoli. On the other hand, Geitler (1932) presents evidence supporting Heitz's theory by finding four nucleoli in telophase stages in a tetraploid form of *Crepis capillaris* which has four satellited chromosomes. It was mentioned in the text that the present author also found some such condition in the species where the satellite situation was thoroughly studied. For example, such correlation was particularly noticed in *Callisia* with two satellites with usually one or two nucleoli; in *Paeonia suffruticosa* in two individual plants with three and four satellites respectively with nucleoli constantly fewer in the former and more in the latter. However, it was found that this correlation was not complete, since it is decisively shown by Van Camp and by the present author that there is no localization of nucleolar development comparable to Heitz's assumption and that, even at resting stages, there are frequently found nuclei that contain nucleoli far above the number expected on the basis of Heitz's theory. It may therefore be pointed out that there are no definite nucleolus-producing chromosomes; that nucleoli may be produced in the form of small globules on the surface of every chromosome, which latter collect into larger globules; that some of these larger globules may come in contact with the satellites and remain attached there; and that finally the number of nucleolar globules may decrease to one by fusion between the globules, so that generally one large nucleolus is found by the time the chromosomes have developed to the late stage of prophase. De Mol's assumption of nucleolar fragmentation is found contrary to all observations made in all species reported here, and by no known mechanism can such a process be adequately explained; on the contrary, the present author observed in living tissue a fusion between two moderately large nucleolar bodies but has never seen any indication of fragmentation.

No adequate explanation can be offered for the origin of a protuberance (bud-like structure) at the side of the large nucleolus in meiotic stages nor for the extranucleolar bodies found both in somatic and

meiotic nuclei. Based on the staining reaction, it is suggested that the extranucleolar bodies may be undissolved nucleolar particles that have been carried by the dividing chromosomes into the new nuclei, there to remain as inert particles. It is difficult to see if there may be a relationship between the bud-like protuberance and extranucleolar bodies. Until the origin of both these bodies is carefully studied from living material, it will be futile to speculate as to their origin and function. Whenever there is a similarity of reaction of nucleolar bodies inside the same nuclear membrane, this may be explained by assuming that each represents a nucleolar globule which, owing perhaps to its being attached to a satellite, is not able to fuse with another such globule into a large single one. The similarity or dissimilarity between the true nucleolus and these bodies may be intelligible if a method of differential staining is applied.

III. Behavior and Function of the Nucleolus. The present author is inclined to put considerable stress on the behavior of vacuolation of the nucleoli and considers this phenomenon of significance, since it was pointed out above that a large vacuole may disappear completely and yet not cause any appreciable decrease in the volume of the nucleolus. Therefore it seems more accurate to assume that instead of the nucleolus being used up as reserve food, it is concerned in the general metabolic processes of the organism as a whole. This assumption may be more validated when we consider that the nucleolus is very probably universally found in all nuclei of all organisms. An exception to this rule is found in the male gamete, as cited by Wilson (1925), Sharp (1926), Tischler (1926), and Belar (1928). This exception is probably more apparent than real, however, for it seems likely that the nucleolus is not lacking but only delayed in its development. The chromosomes for a long time remain unmodified until the male gamete reaches the female gamete, and there they seem to go through a resting stage, during which time nucleoli in small globules come to appear. This conclusion is drawn from the figure presented by Wylie (1923) of *Vallisneria spiralis* and from the studies of Sax and Edmonds (1933) and O'Mara on *Lilium* (1933).

Montgomery (1898) makes the statement that the nucleolar vacuoles in *Spirogyra* are normal structures. It was found by the present author that the nature of these vacuoles does not indicate any peculiarity characteristic only of that species but a similarity in behavior comparable to nucleolar vacuoles observed in *Callisia* stigma hairs and in all other parts of the plant. The number of nucleoli varied from a few small ones, differing among themselves in size, to a single large one. It was

also noticed that thinly opened fine threads of the chromosomes surround the nucleolus, giving an appearance similar to the nucleus illustrated in Fig. 18. Conard (1931) seems to be justified in opposing some investigators who claim the origin of the chromosomes from the nucleolus. To this category may belong the findings of Kater (1928) and Faulkner (1929), who claim that nucleoli give rise directly to chromosomes.

In this connection some *Spirogyra* were treated with Ehrlich-Biondi stain which was introduced under the cover glass, and there was seen a distinct demarcation between the chromosome threads and the nucleolus. The chromosomes did not take any stain, while the nucleolus took a brick red color. It was also observed that the pyrenoids surrounded by the chloroplastic mass took a stain identical with that of the nucleolus. The similarity of staining reaction of the nucleolus and the pyrenoids may signify that they are composed of the same chemical material, or it may merely be a reaction due to their having the same electrical charge. The significance of their similarity in reaction with this stain will remain an open question until their chemical make-up is understood; therefore it is futile even to suggest that nucleoli and pyrenoids may have a homologous function, one in the nucleus and the other in the cytoplasm.

As mentioned in the introduction, according to de Mol (1926), Zirkle (1928), and Fikry (1930), the nucleolus may play a part in transmitting hereditary stimuli from the chromosomes to the cytoplasm. This theory may be justified from the fact that the nucleolus is built up from small globules that may either be exudation products from the chromosomes or formed on the surface of the chromosomes from a chemical combination between some material produced from the metamorphosing chromosomes during the late telophase or between matrix substance and material taken up from the nuclear-sap. It is shown that this "compound" product, the nucleolus, seems to stay unchanged in volume during the entire development of the nucleus, disappearing in the cytoplasm only after the nuclear membrane has vanished, or perhaps when it has gradually become freely permeable to cell-sap. The fact remains that the nucleolus disappears in the cell-sap either completely or partially, and in some cases, as in *Callisia* (Figs. 15-17), *Polystichum* (Fig. 46), and *Yucca* (Fig. 56), it may be left, in its entirety, in the cytoplasm and stay there undissolved, at least for a long time; while in other cases, as was indicated above, the nucleolus may not be dissolved in the cytoplasm at all, or may be included in the daughter nuclei, as seems to be the case in some Protista.

If the assumption is correct that the nucleolus is a compound product

originating, in part, from all the chromosomes and thus containing material from all the chromosomes, and hence from all the genes, and since, excepting perhaps in the case of some Protista, this substance is dissolved in the cell-sap during or after cell division, the suggestion of the above authors that the nucleolus may play a part in transmitting hereditary stimuli from the chromosomes to the cytoplasm deserves consideration. It may be assumed that the chromosomes fundamentally as self-perpetuating bodies divide up and are carried into, and become the basis of the daughter nuclei; that the chromosomes, as such, do not directly transmit gene stimuli, but that this transmission takes place, indirectly, through the matrix substance, which may be considered a by-product of the chromosomes. If this is correct, an explanation may be offered that would perhaps help in interpreting some data presented by Riley (1932) concerning the phenomenon of self-sterility in *Capsella*. The data of this author seem to indicate that a self-sterility factor, instead of exerting its inhibitory influence over only half the number of the pollen grains, as is the case in *Nicotiana* (East, 1929), affects all the pollen grains. In the case of *Nicotiana* the factors of self-sterility may not be so inhibitory as in *Capsella;* hence the difference in these two genera. Therefore a hereditary factor of self-sterility may be transmitted through the nucleolus into the cytoplasm of the microsporocyte and through the four pollen grains resulting from the division of the microsporocyte.

The presence of a bud-like protuberance on the nucleolus in rice meiotic cells has led Selim (1930) to assume that a single nucleolus buds off a secondary nucleolus which disappears during the development of the chromosomes and the primary one at late diakinesis. He supports the view that the secondary nucleolus contributes material to the chromosomes and the primary one to the spindle formation. Budding is taken by him as a separation of the nucleolus into two different materials. Sęthi (1930) has entertained principally the same ideas as Selim. In general, the transportation theory seems to have many followers, based on the fact that there has been found some sort of direct connection between some chromosomes and the nucleolus, and also because there has been observed a difference in stainability of chromosomes in early and late stages, a faint stain, or none, being taken in early stages and a deep stain at late stages. With this is associated the disappearance of the nucleolus by the time the chromosomes are arranged on a metaphase plate. Harper, as early as 1905, working on certain mildews, refuted the presence of direct contact between the chromosomes and nuleolus. Fikry (1930), working with *Rumex*, suggests that the connection observed between nucleolus and spireme is

due to chance, and that there is no constant and fixed connection between them. The present author found no connection between the nucleolus and chromosomes in *Yucca* (Fig. 55); in *Callisia* and others the connection is only between certain chromosomes and the nucleolus.

It was asserted above that the diminution or disappearance of the nucleolus had no correlation either with the stainability of chromosomes or with building up of the chromosome mass. For this adequate explanations were offered.

The theory of the formation of the spindle by one of these nucleoli must be refuted because many investigators have observed and reported that nucleoli may be in part or completely extruded from the cytoplasm, but the chromosomes nevertheless divide, as illustrated in Fig. 56, without the assistance of the nucleolus and move toward opposite poles.

In this connection should be discussed the question of the electromagnetic quality of the nucleolus and chromosomes proposed in the following words by Zirkle (1928) as an addition to a number of theories proposed earlier by others: "The plastin, being electro-positive, changes the electro-negative spireme by flowing into it, to an electro-positive chromatin complex; thus the chromatin, which had collected at the equatorial plate as far as possible from the poles of the spindle, reverses its motion with its electrical charge and migrates to the two poles." Christoff and Gentscheff (1932) have subjected Zirkle's proposed point to a test by putting an electrical charge through a maize sprout, and find that nucleoli do show a definite charge, thus giving support to the above idea. However, it was mentioned early that in living material the present author has observed two large bodies of nucleoli fused together into one, that the nucleolus may be bodily extruded into the cytoplasm without an appreciable decrease in size, as is shown for *Yucca* in Fig. 56. Therefore, based on these two facts alone, the polarity phenomenon can not be responsible for dividing the chromosomes, because two similarly charged bodies ordinarily could not fuse into one, but would repel each other, and because in *Yucca* at least, the nucleoli being extruded into the cytoplasm, they are eliminated from playing any part in the division phenomenon.

It was stated above that the present writer is inclined to put considerable stress on the behavior of vacuolation of the nucleoli. It is very likely that the nucleolus may play a part in the organization of the cell as an organ that may primarily be concerned with assimilative processes by "imbibing" substances from the surrounding medium, converting them into material that may be utilized by the chromosomes especially for their growth and perpetuation. Schaede (1929) states

that "Directe Verbindung zwischen Kernfaden und Nucleolus in dem Sinne, dass ersterer in amöboide Fortsätze des letzteren einmünde, besteht nicht, auch kein unmittelbarer Übergang von Nucleolarsubstanz in den Kernfaden," and that "In Anbetracht ihrer gegenseitigen Beziehungen ist eine Abgabe von Substanz in abgeänderter Form aus dem Nucleolus in den Kernfaden wahrscheinlich . . ." As Schaede points out, the nucleolar substance itself can not be directly involved in this process, as Van Camp and many others have believed. It was amply shown that the nucleolar body remains undiminished during the chromosome development. Its assistance in this process may be of an indirect nature during the vacuolation and devacuolation described above. The latter phenomenon may not be abrupt but simultaneous, since no appreciable decrease or increase was observed during this process.

G. SUMMARY

1. Because of the evidence offered that the chromosomes never lose their individuality in structure and position inside the nuclear membrane, it is argued that a direct flow of any nucleolar substance from one end of a chromosome through the entire spireme is not possible. In the case of *Callisia* some, but not all, chromosomes may be attached to a nucleolus. Applying a delicate differential stain (Ehrlich-Biondi), there was never found an intermediate staining at any point of any chromosome that may have come in contact with the nucleolus, and at all stages the chromosomes were stained pale blue and the nucleolus bright red, indicating that no direct interchange of material had taken place between chromosomes and nucleolus.

2. No correlation was observed between a decrease of nucleolar volume and the process of "emerging" chromosomes. The volume of nucleolus in meiosis studied in some species, once the maximum is reached during late telophase-early leptotene, remains constant all through the chromosome development.

3. The satellite and secondary constriction phenomena in *Callisia*, *Paeonia*, and *Pinus* are analyzed to determine the nature of satellite-nucleolar relationship. The nucleolus is found to originate on the surface of the chromosomes during telophase in the form of small globules, as Van Camp has shown, and not on a specific region (achromatic threads of satellite and secondary constriction) of satellite chromosomes, as Heitz believes; hence there were found numerous globules instead of a limited number corresponding to the number of satellites or segmented chromosomes. When there was a connection between a satellite and a nucleolus, it was found that the nucleolus is attached at

the side of the satellite instead of forming a collar around the satellite achromatic thread.

4. There was found some correlation between the number of satellites and nucleoli, but not exactly as was expected according to Heitz's theory. The symmetry between two sister nuclei in the number, size, and position of nucleoli is suggested to be due primarily to a physical relationship between a satellite and a nucleolus, and to corresponding spaces between the same set of chromosomes in the sister nuclei where nucleolar globules "exuded" from the adjacent chromosome surfaces fill in.

5. A behavior of extrusion of nucleolar particles in the species studied is described. In *Callisia* there were found small extranucleolar bodies which showed a difference in stainability when compared with the nucleolus. Their origin could not be determined. It is suggested that they may be inert nucleolar particles representing pieces of nucleoli that have been retained in the sister nuclei as lagging particles. On the side of the meiotic nucleolus there was often observed a protuberance, a bud-like growth. Its nature and relationship, if any, with some of the free nucleolar bodies observed in meiotic nuclei could not be determined.

6. A critical analysis is presented of de Mol's finding that the size and number of nucleoli vary proportionately as the number of chromosomes is increased in polyploid series. No significant correlation was found in a similar relationship studied in *Petunia*.

7. The results of some observations made from living tissue (stigma hairs of *Callisia*) are as follows: (a) A clear area around a nucleolus is an artifact which can be produced by applying toxic fluids to stigma hairs in culture. (b) Small extranucleolar bodies were often observed in the nuclei of the hairs. The nucleolus was found showing some affinity to these bodies when a clear area was artificially created. (c) No general chromosome and nucleolar connections were observed. The connections, when present, may be limited to certain chromosomes. Often specific connection was found between the nucleolus and the extranucleolar bodies. (d) The vacuole in the nucleolus is normal and not an artifact and probably appears and disappears normally. The phenomenon suggestive of vacuolation is considered by the present author to be of physiological significance. Some data are presented concerning this behavior.

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EXPLANATION OF PLATES 64 TO 67

All sections are longitudinally cut.

Figs. 1–28 *Callisia repens*

Fig. 1. Method: Lewitzky fixation; stained in iron-alum-haematoxylin. Stage: prophase, oblique view, nucleolus in center, one probable attachment between nucleolus and chromosome.

Fig. 2. Method: same as in Fig. 1. Stage: prophase, side view (nucleolus not shown).

Fig. 3. Method: Lewitzky + 0.25% saponin; stained in i.a.h. first; destained and restained in Ehrlich-Biondi mixture. Stage: prophase drawn on nucleolar level, chromosomes stained homogeneously pale blue and nucleolus bright red.

Fig. 4. Method: same as in Fig. 3. Stage: metaphase with two nucleolar pieces.

Fig. 5. Method: same as in Fig. 3, before destaining. Stage: metaphase, the satellite chromosome pair, fully split; one of the sister chromosomes on the left shows a further split.

Fig. 6. Method: same as in Fig. 3. Stage: late anaphase, chromosomes homogeneously pale blue.

Fig. 7. Method: same as in Fig. 5. Stage: an anaphase chromosome with split and twist.

Fig. 8. Method: Lewitzky fixation; stained in crystal-violet-iodine. Stage: split and twisted chromosomes from late anaphase.

Fig. 9. Method: same as in Fig. 3. Stage: telophase; diagrammatic presentation of nucleolar globules.

Fig. 10. Method: same as in Fig. 3. Stage: telophase; two small nucleoli shown.

Fig. 11. Method: same as in Fig. 3. Stage: telophase, with one nucleolus larger than in Fig. 10.

Fig. 12. Method: same as in Fig. 1. Stage: interphase, with three nucleoli (the nucleoli were tested with E.-B. stain; all took bright red color).

Fig. 13. Method: same as in Fig. 1. Stage: dormant stage nucleus in stigma hair, a vacuolated nucleolus with clear area around and four extranucleolar bodies.

Fig. 14. Method: 1 3 minutes Carnoy solution, followed by Lewitzky fixative; stained in c.v.i., destained and restained in E.-B. Stage: prophase from a cell of microsporogenous tissue with two nucleoli drawn from the level of the nucleoli.

Fig. 15. Method: same as in Fig. 14. Stage: prophase of two microsporocyte sister cells, each with an extranuclear nucleolus, drawn from the level of the nucleoli.

Figs. 16 and 17. Method: same as in Fig. 14. Stage: late anaphase of a microsporogenous cell division, with median and polar lagging nucleoli, respectively.

Fig. 18. Method: Fixative same as in Fig. 14; stained in i.a.h. Stage: early leptotene, with a normal nucleolus inside the nucleus and one lagging nucleolus outside.

Fig. 19. Method: same as in Fig. 14. Stage: leptotene, with two small normal nucleoli, one with a small vacuole.

Fig. 20. Method: same as in Fig. 14. Stage: leptotene, with a large normal nucleolus.

Fig. 21. Method: 1-3 minutes Carnoy, followed by Zirkle (1928) No. 4; stained in i.a.h.; later destained and restained in E.-B. Stage: pachytene, one large normal nucleolus with chromosome attach-

ment and two extra smaller nucleoli which stained somewhat differently. (Some of these nucleoli were in the form of a bubble with a very thin membrane stained a somewhat lighter color; hyaline inside.)

Fig. 22. Method: same as in Fig. 21. Stage: pachytene, with a bud-like protuberance at the side of the nucleolus; a satellite chromosome attached to the nucleolus.

Fig. 23. Method: same as in Fig. 21. Stage: Pachytene, with one outside and one inside additional nucleoli, which are of the same nature as those indicated in Fig. 21.

Fig. 24. Method: 1-3 minutes Carnoy, followed by Lewitzky; stained in i.a.h. Stage: pachytene; nucleolus with four small vacuoles, dark stain in the nucleolar substance, somewhat hyaline in the marginal portion.

Fig. 25. Method: Lewitzky fixation; stained in i.a.h. Stage: diakinesis; at the side of the nucleolus a satellite (?) attached. (Chromosome pair shown in outline.)

Fig. 26. Method: same as in Fig. 25. Stage: interphase of first meiotic division, showing globular nucleoli. (Chromosomes could not be shown because of their very faint color; however, these globules were very closely associated with the chromosome threads.)

Fig. 27. Method: same as in Fig. 25. Stage: microspore prophase with a large nucleolus with one probable chromosome attachment, and an extranucleolar body below a long doubled-up chromosome.

Fig. 28. Method: same as in Fig. 25. Stage: microspore metaphase, with one satellite chromosome and an extranucleolar body near the distal end of the same.

Figs. 29 and 30 *Paeonia suffruticosa* (Arboretum plant)

Fig. 29. Method: Lewitzky fixation; stained in c.v.i. Stage: metaphase; a part of a satellite chromosome to which a small piece of nucleolus is attached.

Fig. 30. Method: same as in Fig. 29. Stage: anaphase; parts of chromosomes which are split and show spiral twists.

Fig. 31 *Paeonia suffruticosa* (greenhouse plant)

Fig. 31. Method: same as in Fig. 3. Stage: telophase, where small bright red globular nucleoli were seen along pale blue chromosome threads.

Figs. 32 and 33 *Paeonia suffruticosa* (Arboretum plant)

Fig. 32. Method: same as in Fig. 29, but destained and restained in E.-B. Stage: telophase, sister nuclei. Small bright red globular nucleoli are presented in a diagrammatic way to show approximate number of these globules.

Fig. 33. Method: same as in Fig. 29. Stage: late telophase, showing a symmetry in two sister nuclei in the number, position, and size of nucleoli. Fine chromosome threads are shown diagrammatically arranged in linear fashion. Four-satellited plant with four nucleoli. The number of nucleoli may be as high as seven and possibly more.

Fig. 34 *Paeonia suffruticosa* (greenhouse plant)

Fig. 34. Method: same as in Fig. 3. Stage: interphase of sister cells, each nucleus of which possesses five nucleoli; no symmetry of size and position. Three-satellited plant with five nucleoli.

Fig. 35 *Paeonia Woodwardii*

Fig. 35. Method: Lewitzky fixation; stained in E.-B. Stage: interphase. Six-satellited plant with nine nucleoli.

Fig. 36 *Paeonia Delavayi alba*

Fig. 36. Method and stage: same as in Fig. 35. Six-satellited plant with number of nucleoli as high as eleven, generally less than this number.

Figs. 37 and 38 *Pinus Strobus*

Fig. 37. Method: Lewitzky fixation; stained in E.-B. Stage: telophase; sister nuclei with numerous small nucleolar globules.

Fig. 38. Method: same as in Fig. 37. Stage: interphase; only nucleoli shown. Ten-secondary-constricted plant with as high as fourteen nucleoli.

Figs. 39-44 *Pinus Thunbergii*

Fig. 39. Method: Lewitzky fixation; stained in E.-B. Stage: early leptotene with nine nucleoli.

Fig. 40. Method: Lewitzky fixation; stained in E.-B. Stage: early diplotene with nine nucleoli.

Fig. 41. Method: same as in Fig. 40. Stage: late diplotene with six nucleoli.

Fig. 42. Method: same as in Fig. 40. Stage: beginning of metaphase with four nucleoli.

Fig. 43. Method: fixation same as in Fig. 40, but stained in c.v.i. Stage: interphase after meiotic division. Only one of the two sister nuclei shown possesses nine nucleoli.

Fig. 44. Method: same as in Fig. 43. Stage: late telophase of tetrad formation, number of nucleoli varying from eight to twelve.

Figs. 45-47 *Polystichum acrostichoides*

Fig. 45. Method: Lewitzky fixation; stained in E.-B. Stage: metaphase, with small nucleolar globules in the division region, and one outside this region, remnant of an earlier cell division.

Fig. 46. Method: same as in Fig. 45. Stage: interphase from periblem marginal region with a large nucleolus inside, bag-like, containing darker staining particles; similar kinds free from the "bag." One large round nucleolar body stained darker like the small ones outside the nucleus, this body being a remnant of an earlier cell division.

Fig. 47. Method: same as in Fig. 45. Stage: a nucleus in early prophase with a single bag like nucleolus containing darker staining smaller particles. This figure is from a more actively dividing region.

Figs. 48 and 49 *Callisia repens*

Fig. 48. Method: Lewitzky fixation + 0.25% saponin; stained in i.a.h. Stage: metaphase plate showing the structural differences of the chromosomes.

Fig. 49. Method: Navashin fixation, half strength; stained in c.v.i. Stage: anaphase.


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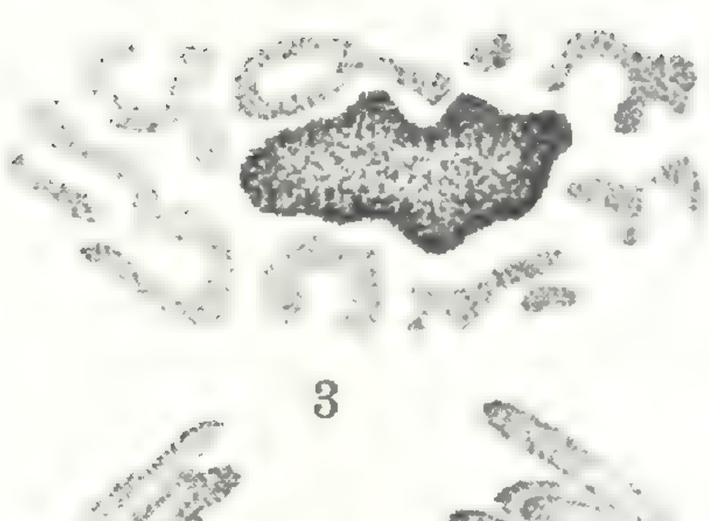
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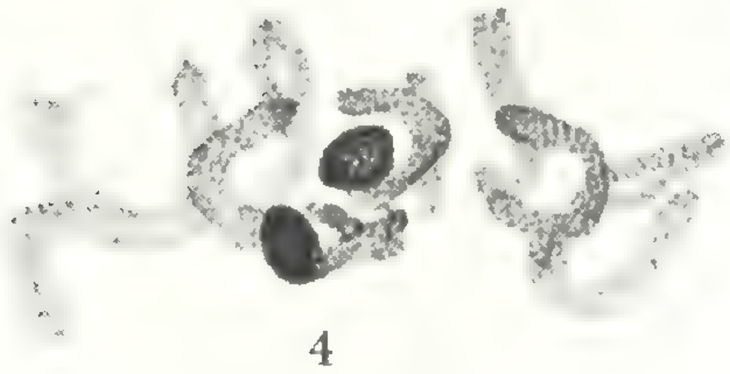
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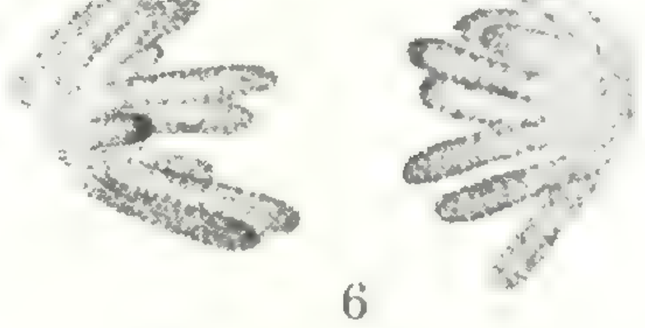
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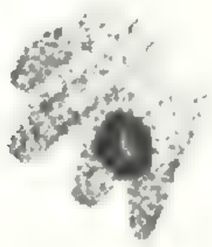
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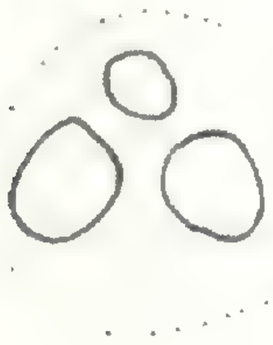
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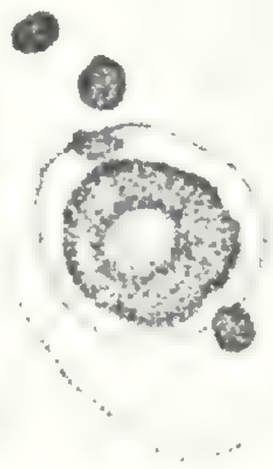
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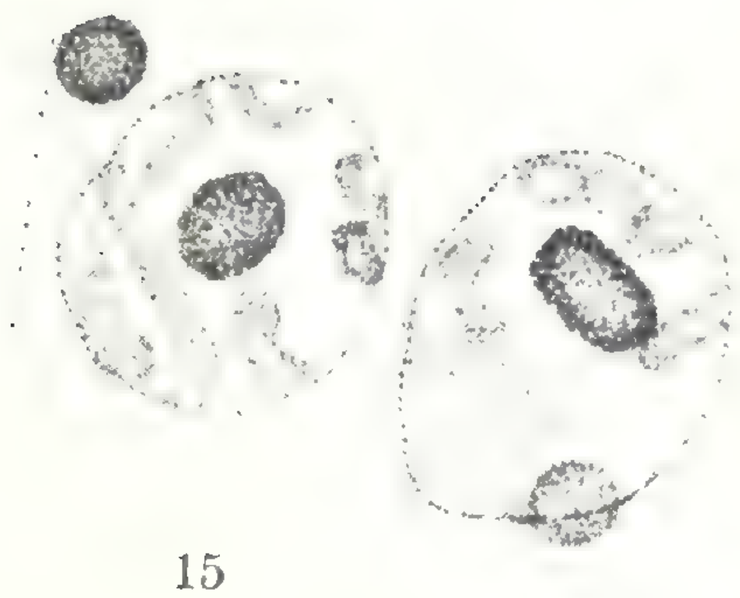
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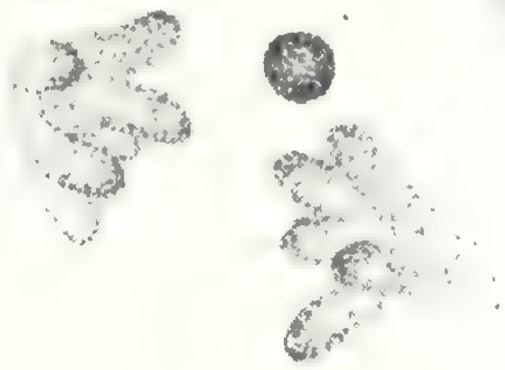
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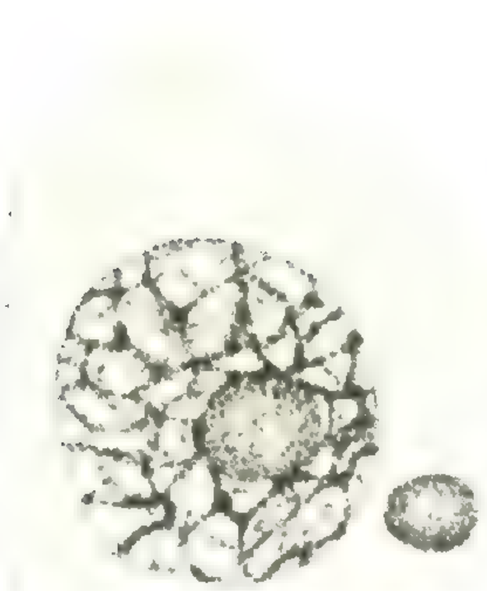
ORIGIN AND BEHAVIOR OF THE NUCLEOLUS IN PLANTS

THE HELIOTYPE CORP. BOSTON



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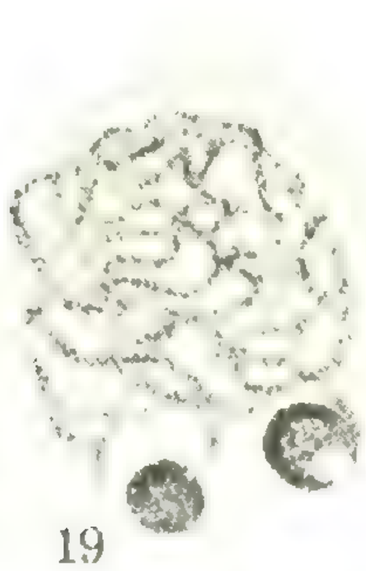

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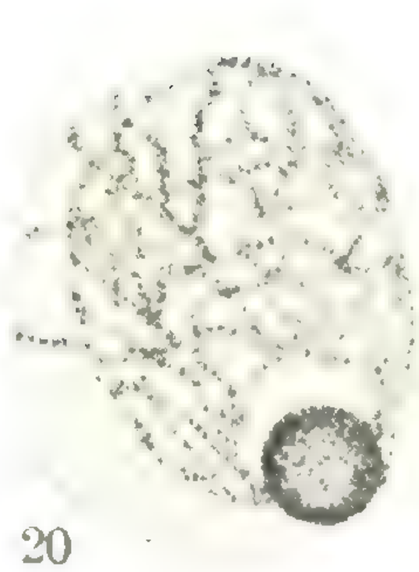

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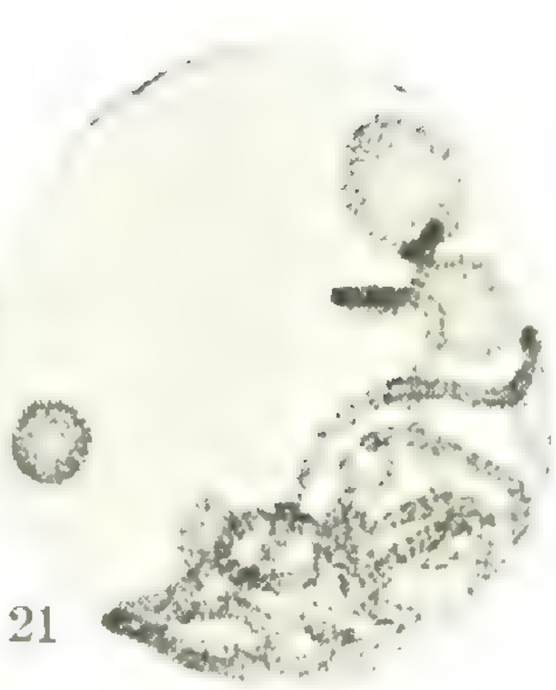

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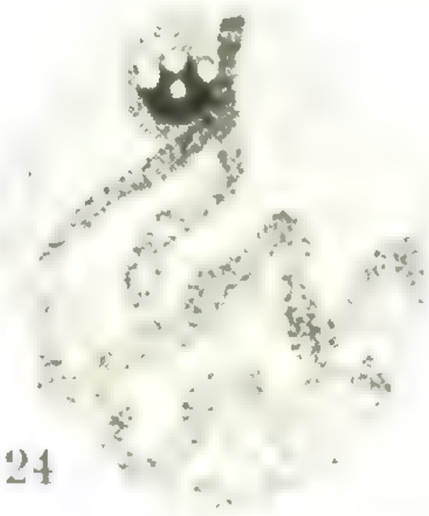

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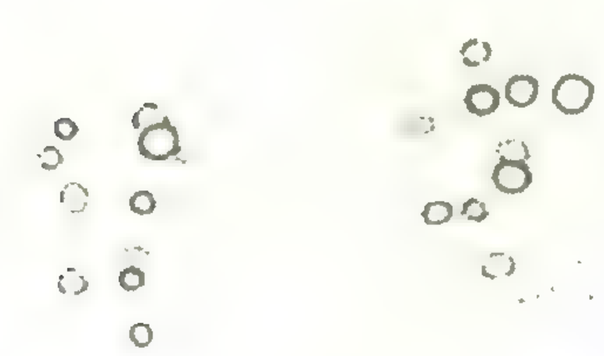

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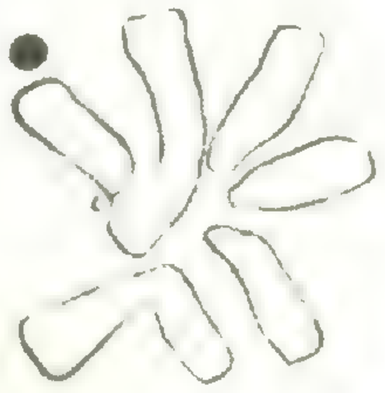

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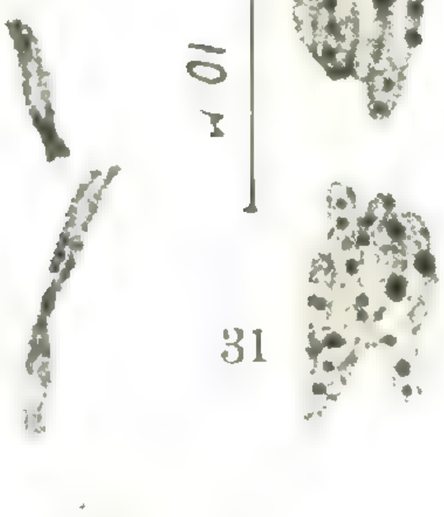

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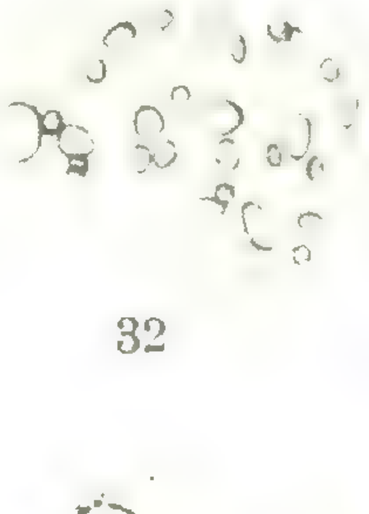

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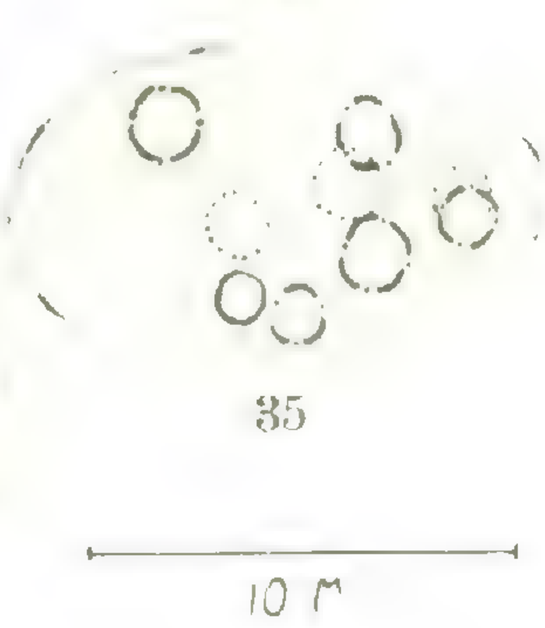

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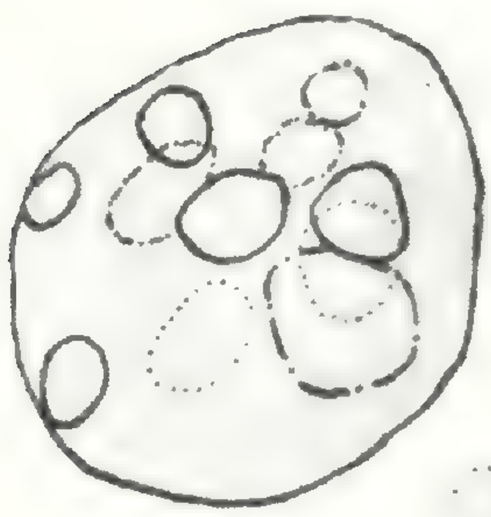
ORIGIN AND BEHAVIOR OF THE NUCLEOLUS IN PLANTS

THE HELIOTYPE CORP. BOSTON



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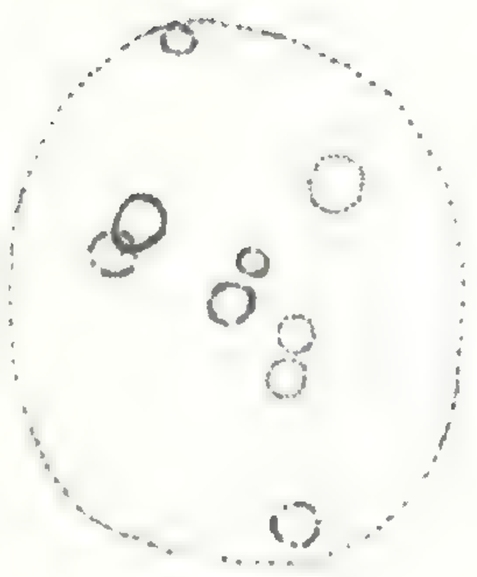

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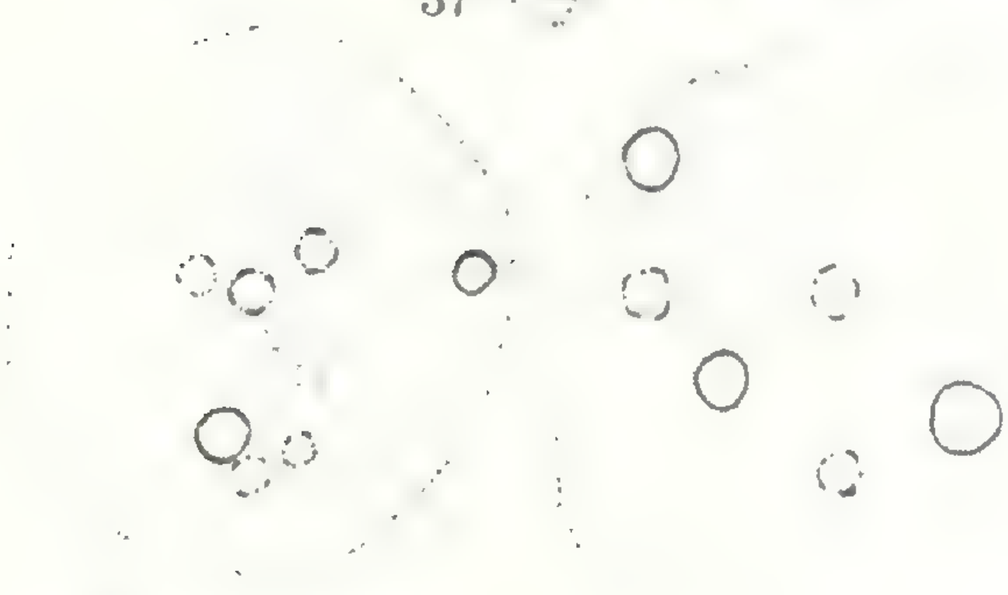

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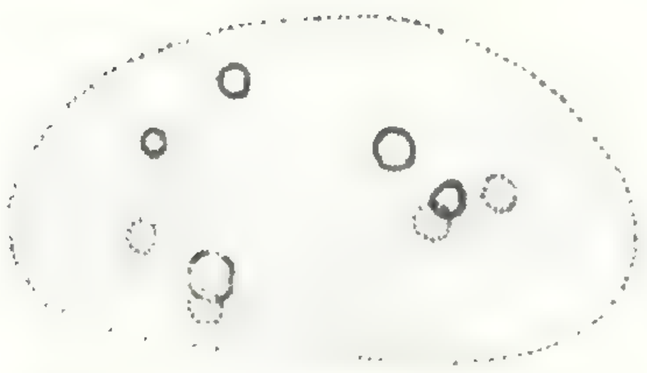

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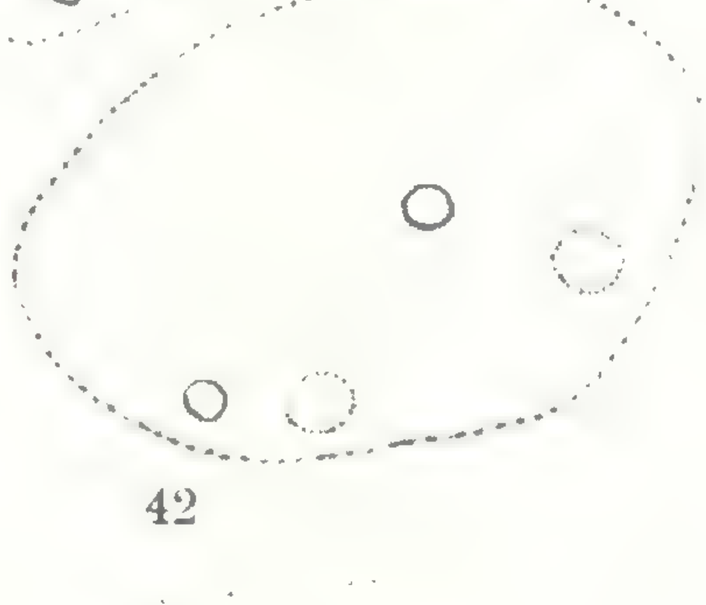

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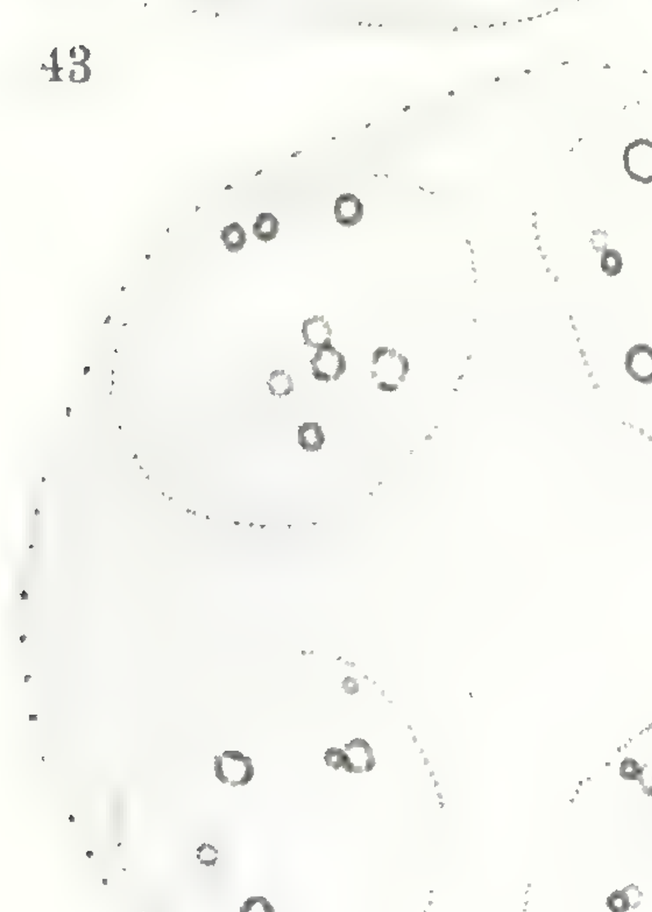

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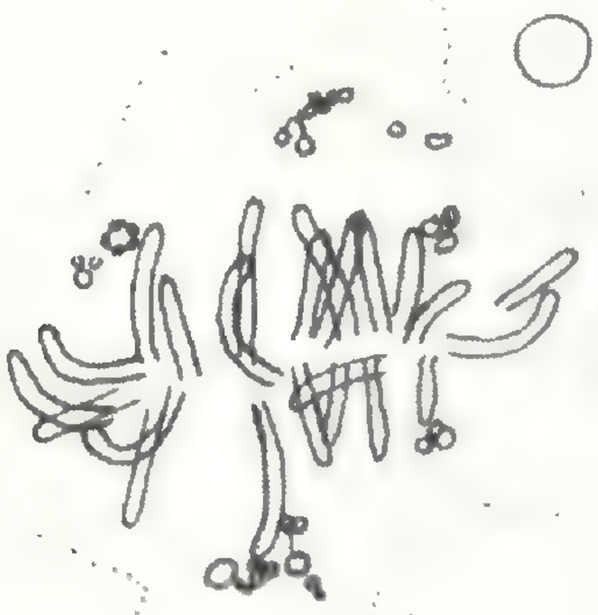

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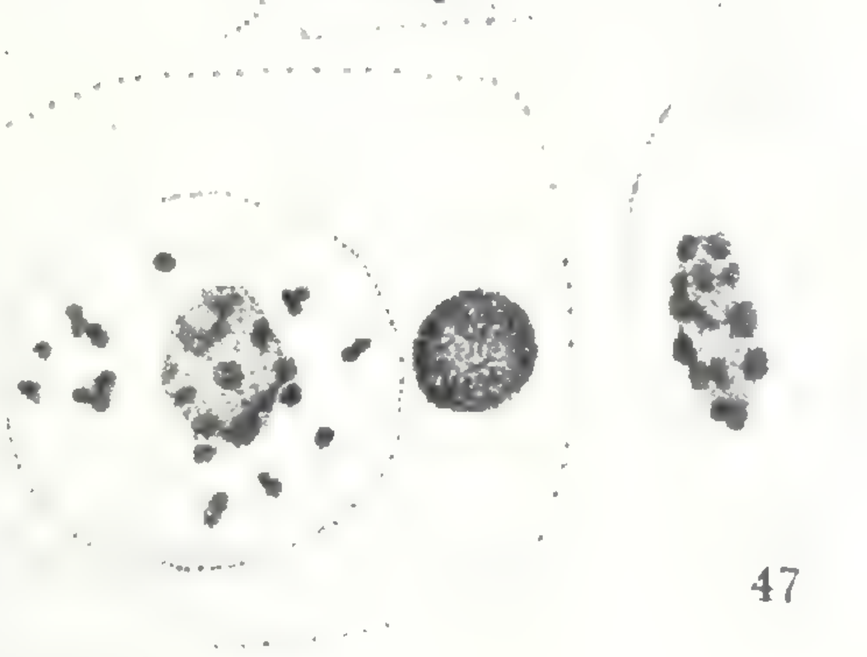

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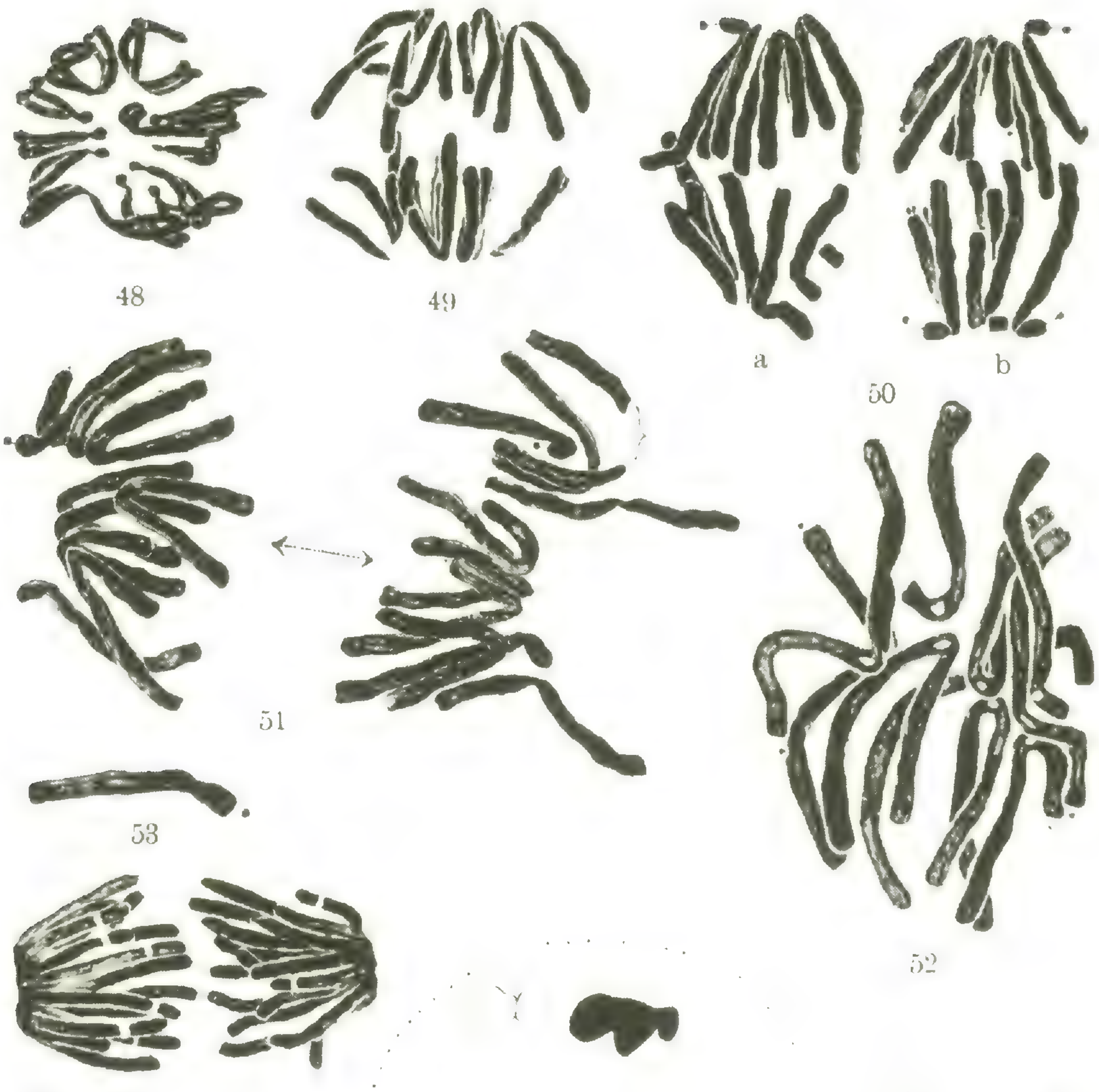
ORIGIN AND BEHAVIOR OF THE NUCLEOLUS IN PLANTS

THE HELIOTYPE CORP. BOSTON



ORIGIN AND BEHAVIOR OF THE NUCLEOLUS IN PLANTS

THE HELIOTYPE CORP. BOSTON

Fig. 50 *Paeonia suffruticosa* (Arboretum plant)

Fig. 50. Method: Lewitzky fixation; stained in c.v.i. Stage: anaphase presented in two sections with four satellites in total.

Fig. 51 *Paeonia suffruticosa* (greenhouse plant)

Fig. 51. Method: same as in Fig. 31, but again restained in c.v.i. Stage: anaphase, pressed flat to show the satellited feature. There are three satellites instead of four; one of the subterminally constricted chromosomes is without one.

Fig. 52 *Paeonia Woodwardii*

Fig. 52. Method: same as in Fig. 35, but restained in c.v.i. Stage: metaphase, side view, pressed flat to show the satellited feature. This plant has three pairs of satellites.

Fig. 53 *Paeonia Delavayi alba*

Fig. 53. Method: same as in Fig. 52. Stage: metaphase, only one chromosome showing. This plant has three pairs of satellites like those shown in Fig. 52, but the satellite, as shown here, is larger than the one on the subterminal chromosomes in Fig. 52, and is attached with a longer achromatic thread.

Fig. 54 *Pinus Strobus*

Fig. 54. Method: same as in Fig. 37, but stained in i.a.h. Stage: anaphase, only part of the chromosomes showing. At most five pairs of secondary constricted chromosomes are present (nine actually were counted).

Figs. 55 and 56 *Yucca flaccida*

Fig. 55. Method: smeared and fixed in Fleming strong; stained in c.v.i. Stage: diakinesis, drawn on nucleolar level. No attachment was observed between any of the chromosomes and nucleolus.

Fig. 56. Method: same as in Fig. 55. Stage: telophase; two attached nucleolar bodies at the upper pole.

Fig. 57 *Hepatica triloba*

Fig. 57. Method: same as in Fig. 55. Stage: two nucleoli are shown diagrammatically, *a* from very early leptotene, and *b* from diplotene. No difference in size is seen.

CYTOLOGICAL LABORATORY, ARNOLD ARBORETUM,
HARVARD UNIVERSITY.

SOME HYMENOMYCETES FORMING MYCORRHIZAE WITH PINUS STROBUS L.

A. B. HATCH AND C. TALBOTT HATCH

With plates 68 to 71

THE CLASSICAL EXPERIMENTS of Elias Melin (1922) yielded the first conclusive evidence that the Hymenomycetes are involved in the mycorrhizal associations of forest trees. The only fully satisfactory means available for exploring such relationships, namely, the artificial association of tree seedlings with fungi in pure culture, was employed.

In earlier experiments (Melin, 1921) mycorrhizal fungi (*Mycelium radicis* subsp.) were isolated from tree roots and their relationships to tree seedlings demonstrated. By 1925 species among the genera *Lactarius, Russula, Cortinarius, Tricholoma, Amanita,* and *Boletus* had been shown to form mycorrhizae with *Pinus, Picea, Larix, Betula,* or *Populus* (Melin, 1922, 1923a, 1923b, 1924, 1925a, 1925b, 1925c). Hammarlund (1923) and Masui (1927) have subsequently reported successful syntheses in pure culture.

Precise knowledge on the etiology of the mycorrhizal habit of American Pines is fundamental to studies of their nutrition.[1] With this larger problem in view, a series of syntheses experiments were pursued with *P. Strobus* L. and *P. resinosa* Ait. in association with species of *Boletinus, Boletus, Lactarius, Russula, Amanita,* and *Mycelium radicis* subsp. cultured in both Sweden and America (See Table 1). The study was initiated in Professor Melin's laboratory at the Mycological Laboratory, Royal Academy of Forestry, Stockholm, in the fall of 1929.

Descriptions of technique used in syntheses experiments have been published by Melin (1921, 1923a, 1925a). But Melin has been so frequently misquoted and his technique so often incorrectly followed that recapitulation in connection with the present work is desirable.

Cultures of known fungi were obtained by means of tissue isolations from the pileus and upper portion of the stipes of young sporophores. The following fungi were obtained in culture:[2]

[1]Absorbing roots (short roots, "Saugwurzeln") of Pines in natural habitats are completely mycorrhizal. Thus nutrients entering the tree by these channels must first pass through a fungal mantle that entirely separates the root cells of the tree from the soil.

[2]*Boletinus porosus* and *Boletus castaneus* were identified by Mr. C. L. Krieger, Washington, D. C., *Lactarius chrysorheus* by the junior author and all other specimens by Professors E. Melin and T. Lagerberg.

Lactarius chrysorheus Fr.
" *deliciosus* (L.) Fr., Plate 68, G.
" *subdulcis* (Bull.) Fr.
Russula fragilis (Pers.) Sing.
" *puellaris* Fr.
Amanita muscaria (L.) Fr., Plate 68, D.
Boletus chrysenteron (Bull.) Fr.
" *piperatus* Bull.
" *granulatus* L., Plate 68, B.
" *luteus* (L.) Fr.
" *castaneus* Bull., Plate 68, F.
" *bovinus* (L.) Fr., Plate 68, E.
" *edulis* Bull.
Boletinus porosus (Berk.) Peck, Plate 68, A.

The plan of the work included syntheses experiments with *Mycelium radicis* subsp. isolated from both American and Swedish conifers. The technique developed by Melin (1921, 1925a) for isolating mycorrhizal fungi was employed. Essentially this consists of: (1) selecting comparatively young and clean long-roots bearing mycorrhizae; (2) washing these thoroughly in a strong stream of tap-water; (3) cutting the roots into short lengths, each bearing one mycorrhiza; (4) surface sterilizing the latter in 0.1 per cent bichloride of mercury; and (5) rinsing in three or more changes of sterile water. The time required for surface sterilizing small mycorrhizae of the forked (*Pinus*) or racemose (*Picea*) types was two to five seconds (Melin, 1923a, p. 125). A considerable number of contaminations were inevitable, but longer treatments are lethal to the true endophytes. For tuberous mycorrhizae of Pine one minute usually did not prove injurious. After rinsing, the pieces were placed either in agar petri dishes or on nutrient liquid or agar in test tubes. Uncontaminated pieces were later transferred to suitable culture media.

All of the culture media employed contained, or consisted of, malt extract (See Melin, 1925a, p. 10). For the more sensitive fungi (obtained from either known sporophores or from roots) 5 per cent malt extract, sterilized by passage through a Berkefeld filter, was a suitable medium. Rapidly growing forms (*Boletus bovinus*, etc.) were cultivated on autoclaved 5 per cent malt extract with 2 per cent agar (See Table 1). In the latter case a minimum pressure and period of sterilization was conducive to rapid growth of the organisms.[1]

[1]The American brands of malt extract experimented with were not suitable. They are apparently evaporated at high temperatures, which destroy some nutritive properties. We have used Liebig's malt extract obtained from Apoteksvarucentral Vitrum, Stockholm.

TABLE 1

DATA ON FUNGAL CULTURES

Fungus	Place collected	Date	Most abundant trees in stand	Diam. growth of colonies in mm.	Color of hyphae	Color change of media	Type of Colony	Remarks
Lactarius chrysorheus	Westtown, Pa.	8/26/29	pure P. Strobus plantation	10–12 (ME)[c]	yellowish white	slight	loose, submerged	
Lactarius deliciosus	Djursholm[a]	9/22/29	Pinus, Betula	30–35 (ME)	yellow to whitish yellow	slight	loose, submerged (ME) aërial, submerged (MA) (Pl. 68)	Cultivated on solid media 4 mos. after isolation. Formed strands in culture with Pine.
Lactarius subdulcis	Djursholm	9/20/29	Pinus, Picea	3–4 (ME)	white	slight	compact, submerged	
Russula fragilis	Djursholm	9/20/29	Pinus, Picea	2–4 (ME)	white	slight	compact, submerged	
Russula puellaris	Djursholm	10/15/29	Betula, Pinus	2–4 (ME)	white	slight	compact, submerged	
Amanita muscaria	Djursholm	9/22/29	Betula	5–7 (MA)[f]	white	slight	compact, submerged (ME) compact, aërial (MA) (Pl. 68)	
Boletus chrysenteron	Experimentalfältet[a]	10/3/29	Quercus	2–4 (ME)	light brownish yellow	slight	compact, submerged	
Boletus piperatus	Djursholm	10/28/29	Pinus, Picea	8–10 (ME)	lemon yellow	dark brownish	loose, aërial	
Boletus granulatus	Djursholm	10/9/29	Pinus, Betula	50–55 (MA)	white, brownish with age	light brownish	loose, aërial with strands	Formed strands when associated with seedlings in pure culture.
Boletus luteus	Djursholm	9/20/29	Pinus, Betula	70–75 (MA)	white, brownish with age	dark brownish	aërial (Pl. 68)	
Boletus castaneus	Ansonia, Pa.	8/24/29	Tsuga, Betula	57–62 (MA)	white	pinkish	aërial even (Pl. 68)	

TABLE 1—Continued

DATA ON FUNGAL CULTURES

Fungus	Place collected	Date	Most abundant trees in stand	Diam. growth of colonies in mm.	Color of hyphae	Color change of media	Type of Colony	Remarks
Boletus bovinus	Djursholm	9/20/29	Pinus, Picea	65–70 (MA)	white, brownish with age	dark brownish	aërial (Pl. 68)	
Boletus edulis	Djursholm	9/20/29	Pinus, Betula	3–4 (ME)[c]	white	none	compact, submerged	
Boletinus porosus[d,e]	Warren, Pa.		Betula, Fagus, Tsuga, Prunus	64–69 (MA)[f]	deep brownish yellow	dark brown to black	crusted on surface of media, margin serrate	Numerous clamp connections
M.r. (Pinus) Strobi 1[e]	Ansonia, Pa.	Apr. '29	Conifer, hardwood	33–37 (MA)	white, brown with age	brownish	aërial	
M.r. (P.) sylvestris 1	Tureberg[a]	9/29/29	Pinus, Picea, Betula	35–40 (MA)	white, brown with age	brownish	aërial	
M.r. nigrostrigosum[b]	Kulbäcksliden[a]	4/2/30	Pinus, Betula, Picea		jet black	none	compact, submerged (ME) compact, aërial (MA)	
M.r. atrovirens 1[e]	Keene, N. H.	4/17/29	Pinus	85–95 (MA)	grayish green	none	See Melin 1923	
M.r. atrovirens 2[b]	Kulbäcksliden	4/2/30	Pinus, Betula, Picea	60–65 (MA)	grayish green	none	See Melin 1923	
M.r. (Picea) Abietis 1	Tureberg	9/29/29	Pinus, Picea, Betula	15–18 (ME) 10–12 (MA)	white-pinkish	brownish black	loose, submerged (ME)	Numerous clamp connections

a. Sweden.
b. Isolated from seedlings grown in soil-sand pot experiments by P. R. Gast. Soil from Brända Holmen, Kulbäcksliden Experimental Forest, Vindeln, Sweden.
c. ME—5 per cent liquid malt extract sterilized by passage through Berkefeld filter. MA—5 per cent malt extract, 2 per cent agar sterilized by autoclaving (Liebig's Malt Extract).
d. Isolated by A. H. Hough, Allegheny Forest Experiment Station. Subsequent isolations made by us in 1932 check exactly with original culture.
e. Cultured on an American brand of desiccated malt extract.
f. Those marked "(MA)" represent growth for 30 days on 30 ml. 5 per cent malt agar in 100 mm. Petri dishes.

In designating the imperfect stages of mycelia isolated from roots we have followed Burgeff (1932, p. 147) and included the generic names (in parenthesis) as well as the specific names of the vascular plants from which the mycelia were isolated. These names are of value to the individual investigator and likewise conveniently serve to inform the reader of the identities of the hosts from which they were isolated. In those cases where the mycelial characters are so marked that the fungus in question may easily be recognized by other investigators (particularly when the fungus is associated with more than one vascular plant), the name of the plant from which it was isolated is of less value and may be substituted by a descriptive specific name. Examples of such fungi are *Mycelium radicis atrovirens* Melin, and *M. r. nigrostrigosum* Hatch.

The following fungi were isolated from mycorrhizae of *Pinus Strobus*, *P. sylvestris* L., *Picea Abies* (L.) Karst.: *Mycelium radicis* (*Pinus*) *Strobi 1*, *M. r.* (*Pinus*) *sylvestris 1*, Plate 68, C, *M. r. nigrostrigosum*, *M. r. atrovirens 1*, *M. r. atrovirens 2*, *M. r.* (*Picea*) *Abietis 1*, Plate 68, I.

The tree seeds used in our experiments were obtained through commercial seed houses. They were soaked over-night in water, surface sterilized for two minutes in 0.1 per cent bichloride of mercury, and rinsed in several changes of sterile water. They were then sown on agar in petri dishes, and contaminations and infected seeds were removed with a sterile spatula as they became evident. Early experience demonstrated that the particular sample of *P. Strobus* seeds we used required an after-ripening treatment to obtain even nominal germination. Barton (1928) had shown that cold storage treatment was effective in hastening germination of Southern Pine seeds. We, therefore, surface sterilized seeds of *P. Strobus* and stored them in a frigidaire at four to ten degrees centigrade for a period of two months. Regardless of the acidity of the media (moist filter paper, agar, and peat) which we varied from pH 3.5 to neutrality, germination was uniformly good (approximately 80 per cent).[1] Germination was procured in a constant temperature room at 25 degrees centigrade. As they germinated, the seeds were transferred directly into the culture chambers.

The culture technique developed by Melin (1925a, etc.) was employed in the syntheses. Fluvio-glacial sand was screened and that portion having particle sizes between 0.5 and 2.0 mm. was used (adequate aëration of the substratum is not possible in an undrained flask if smaller particles are included). The sand was boiled in concentrated

[1]Barton (1930) has published a second paper in which success is reported with low temperature treatments of *P. Strobus*.

hydrochloric acid for two hours, washed several hours in running tap-water, and finally in five changes of distilled water. It was dried in an oven, and 150 gram samples were weighed into 300 ml. Erlenmeyer flasks. The nutrient solution added to this substratum contained the following:

KH_2PO_4	0.5 grams
$CaCl_2$	0.05 "
NaCl	0.025 "
$MgSO_4 + 7\ H_2O$	0.15 "
$(NH_4)_2PO_4$	0.25 "
Iron citrate	0.025 "
Dextrose	0.5 "
Distilled water	1. litre

Thirty-seven ml. of this solution were added to each flask. The pH of the solution was 6.57. After autoclaving with the sand, this changed to approximately 4.2.

The nutrient conditions in our experiments differed from those in Melin's (1923a, p. 159). We used a less concentrated solution and likewise added 37 rather than 50 ml. of solution to each flask. Further, the growth of *P. Strobus* is considerably greater in culture than that of *P. sylvestris* seedlings. The differences in the nutritional conditions of the two sets of Pines were, therefore, quite marked. Since $\frac{\text{root}}{\text{shoot}}$ growth is greater when nutrients are present in comparatively small quantities, it was argued that more short roots would develop. The probability of obtaining mycorrhizae would therefore be enhanced. It is not necessary, however, to obtain large numbers of mycorrhizae in syntheses experiments, as has been emphasized by McArdle (1932, p. 314). The unquestionable demonstration of the presence of only one typical mycorrhiza is adequate proof that the organisms concerned enter into mycorrhizal association with each other.

The assembled culture chamber consisted of the Erlenmeyer flask with an inverted beaker over the cotton plug (Plate 70, D). Between March 23, 1930 and April 9, 54 of these units, each with a germinated seed of *P. Strobus*, and 52 units with *P. resinosa* seedlings, were set up. They were placed in the diffused light of a west window and received direct solar radiation, passing through the foliage of a large oak tree, late in the evening only (Plate 70, D). On June 5th to 7th the seedlings were inoculated with all of the fungi listed above. The sub-cultures from which inoculations were made were less than ten days old. A number of seedlings were reinoculated August 21.

Half of the flasks were opened during November 1930. These were

chiefly *P. Strobus* syntheses.[1] The remainder were kindly cared for by Professor Melin until the fall of 1931, when the seedlings were placed in fixing solution and shipped to the authors in America. Except for the latter, the substrata in all flasks were tested for contaminations at the close of the experiment by placing sand from the flasks on malt agar media in culture tubes. Of thirty-five cultures of *P. Strobus* opened in November 1930, three were contaminated. A number of contaminations from seed-coat infections with *P. resinosa* were observed during the course of the experiment. Similar contaminations with *P. Strobus* did not occur, since these seeds were on moist agar for nearly three months before they were transferred to the flasks and, consequently, seed-coat infections were eliminated.

Fixation was with Karpchinko solution; it did not prove particularly good for mycorrhizal details. The roots were embedded in paraffin for sectioning; both the butyl and ethyl alcohol series were used. Gross photographs of mycorrhizal roots were made in distilled water between glass plates. Microplanar or Tessar lenses, and Ilford panchromatic soft gradation plates with a Wratten B (red) filter were used. The staining technique employed will be reported by Dr. K. D. Doak in a future communication.

RESULTS

Typical ectotrophic mycorrhizae were form with *P. Strobus* by twelve of the fungi investigated:

Lactarius chrysorheus Fr.,	Plate 70, E
" *deliciosus* (L.) Fr.,	" 69, A; Plate 71, A
Amanita muscaria (L.) Fr.,	" 69, C; " 71, D
Boletus castaneus Bull.,	" 70, I
" *bovinus* (L.) Fr.,	" 70, F
" *luteus* (L.) Fr.,	" 69, B
" *granulatus* L.,	" 70, H
Boletinus porosus (Berk.) Peck,	" 70, G; Plate 71, C
Mycelium radicis nigrostrigosum	
" " (*Picea*) *Abietis* 1	Plate 71, B
" " (*Pinus*) *Strobi* 1	
" " (*Pinus*) *sylvestris* 1	

The mycelia of the *M. r. atrovirens* type, as in Melin's experiments, overgrew the aërial parts of the seedlings and failed to exhibit any indications of mycorrhiza-formation (Plate 70, A). The mycelia

[1] *P. resinosa* grew very poorly in the medium used in these experiments. Root development was adequate for mycorrhiza-formation in only two or three cases. These will be reported elsewhere.

of all other fungi failed to develop in the substrata, and information on their ability to form mycorrhizae was not obtained.

DISCUSSION

Positive results with *Boletinus porosus* adds *Boletinus* to the genera of fungi that have been proved to contain mycorrhiza-forming species. *Lactarius chrysorheus* and *Boletus castaneus* are also added to the list of known mycorrhizal organisms. The remaining fungi (with the exception of those isolated from tree roots) have previously been tested in pure culture with success as follows:

Lactarius deliciosus, with *Pinus mugo* Turra (*P. montana* Mill.), *P. sylvestris* and *Picea Abies* (Melin, 1924, 1925a).

Amanita muscaria, with *Betula pendula* Roth, *B. alba* Roth, *Larix decidua* Mill. (*L. europaea* DC.), *Pinus sylvestris*, and *Picea Abies* (Melin, 1923a, 1925a).

Boletus granulatus, with *P. sylvestris* and *P. mugo* (Melin, 1923a, 1924b).

Boletus bovinus, with *Pinus densiflora* Sieb. & Zucc. (Masui, 1927).

Boletus luteus, with *P. sylvestris*, *P. mugo*, *Larix decidua* Mill., *L. occidentalis* Nutt. and *Picea Abies* (Melin, 1923a, 1923b, 1925a).[1]

Concerning *Amanita muscaria*, Melin (1925a, p. 100) reports: "Der Pilz scheint aber den Pflanzen gegenüber eine ziemlich hohe Virulenz gehabt zu haben." A similar tendency was exhibited by our culture and by *Lactarius chrysorheus* in a 6-months synthesis as exemplified by the development of a heavy intercellular net. A culture plant removed after 15 months' association with *A. muscaria*, on the other hand, showed normal infection (Plate 69, C).

Concerning *Boletus bovinus*, Masui (1927) inoculated seedlings of *Pinus densiflora* growing in large test tubes on nutrient agar and reports that the seedlings were killed by this fungus. In Erlenmeyer flasks the fungus grew up the stem of the seedlings (l. c., p. 203 and Plate XI., Fig. 7). We note that in the latter photograph the plugs of the flasks

[1]Masui (1927) conducted syntheses experiments between *Pinus Thunbergii* Parl., *Quercus myrsinaefolia* Bl., *Q. phillyraeoides* Gray, *Q. glauca* Thunb., *Q. mongolica* Fisch. var. *grosseserrata* Rehd. & Wils., *Q. paucidentata* Franch., and a fungus which he isolated from the tuberous (compound) mycorrhizae of the last named Oak. He believed these mycorrhizae were formed by the mycelium of a *Boletus*, the sporophore of which was attached to the mycorrhiza from which isolations were made. He listed this sporophore as *Boletus luteus* (?). In a footnote (1927, p. 195) Masui mentions that Dr. Krieger believes the specimen may be *B. granulatus*. The identity of the culture is, therefore, not certain, and we have excluded these results from the list above, which represents authentic pure culture syntheses only.

were covered. The explanation of the behavior of *B. bovinus* as reported by Masui is, therefore, probably attributable to excessively high humidities. Our culture of *B. bovinus* grew very rapidly, and quickly covered the substrata within the flasks (the only fungus that covered the sand with surface growth), but exhibited no tendency to overgrow the seedlings (Plate 70, C). The mycorrhizae formed by *B. bovinus* possessed hyphal mantles one-fourth of their total thickness.

It is of interest to record here that in some of the cultures two easily separable fungi, *Mycelium radicis nigrostrigosum* and *M. r.* (*Picea*) *Abietis 1*, were associated with the same seedling. Typical mycorrhizae were formed by each of the fungi with short-roots, and in addition *M. r. nigrostrigosum* formed a secondary mantle over several of the mycorrhizae of the *M. r.* (*Picea*) *Abietis* type (Plate 71, B).

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EXPLANATION OF PLATES

Plate 68. Colonies of mycorrhiza-forming fungi grown in 100 mm. Petri dishes on 30 ml. of 5 per cent malt extract (Liebig's) agar for 30 days (60 days for D and 20 days for F) at 25 degrees centigrade, × 1.

A. *Boletinus porosus.*
B. *Boletus granulatus.*
C. *Mycelium radicis (Pinus) sylvestris 1.*
D. *Amanita muscaria.*
E. *Boletus bovinus.*
F. *Boletus castaneus.*
G. *Lactarius deliciosus.*
H. *Mycelium radicis (Picea) Abietis 1.*

Plate 69. Mycorrhizae on roots of *Pinus Strobus* seedlings grown in pure culture.

A. Whole root system of seedling inoculated with *Lactarius deliciosus.* All short roots mycorrhizal, × 4. (Photo, U. S. Dept. Agric.)
B. Mycorrhizae formed by *Boletus luteus*, × 9.
C. Mycorrhizae formed by *Amanita muscaria*, × 9.

Plate 70. A. Seedling of *Pinus Strobus* inoculated with *Mycelium radicis atrovirens 2*, showing mycelial growth on stem and lower leaves, × 2/3. (Top of flask removed for photo.)
B. Uninfected short roots of *P. Strobus.* Seventeen months old seedling, × 4.
C. Seedling of *P. resinosa* inoculated with *Boletus bovinus*, showing mycelial growth over sand substratum, × 2/3. (Top cut from flask for photo.)
D. Cultures of *P. Strobus* and *P. resinosa* in west window of the Royal Academy of Forestry, Stockholm.
E. Mycorrhizae of *P. Strobus* formed by *Lactarius chrysorheus*, × 6. (Photo, U. S. Dept. Agri.)
F. Mycorrhizal short roots of *P. Strobus* formed with *Boletus bovinus*, × 9.
G. Mycorrhizae of *P. Strobus* formed with *Boletinus porosus*, × 8.

H. Mycorrhizal roots of *P. Strobus* formed with *Boletus granulatus*, × 6.
I. Mycorrhiza of *P. Strobus* formed with *Boletus castaneus*, × 10.

Plate 71. Photomicrographs of mycorrhizae of *P. Strobus* from syntheses experiments, showing distribution of mycelia between the cortical cells, and mantle structure.
A. Inoculum—*Lactarius deliciosus*, medial, longitudinal section, × 400. (Photomicrograph by K. D. Doak.)
B. Inocula—*Mycelium radicis* (*Picea*) *Abietis 1* and *M. r. nigrostrigosum*, somewhat oblique, longitudinal section, × 400. *M. r. nigrostrigosum* forming a secondary mantle over that of *M. r.* (*P.*) *Abietis 1*. (Photomicrograph by K. D. Doak.)
C. Inoculum—*Boletinus porosus*, medial, longitudinal section, × 370.
D. Inoculum—*Amanita muscaria*, oblique, transverse section, × 460. From 17-months old seedling.

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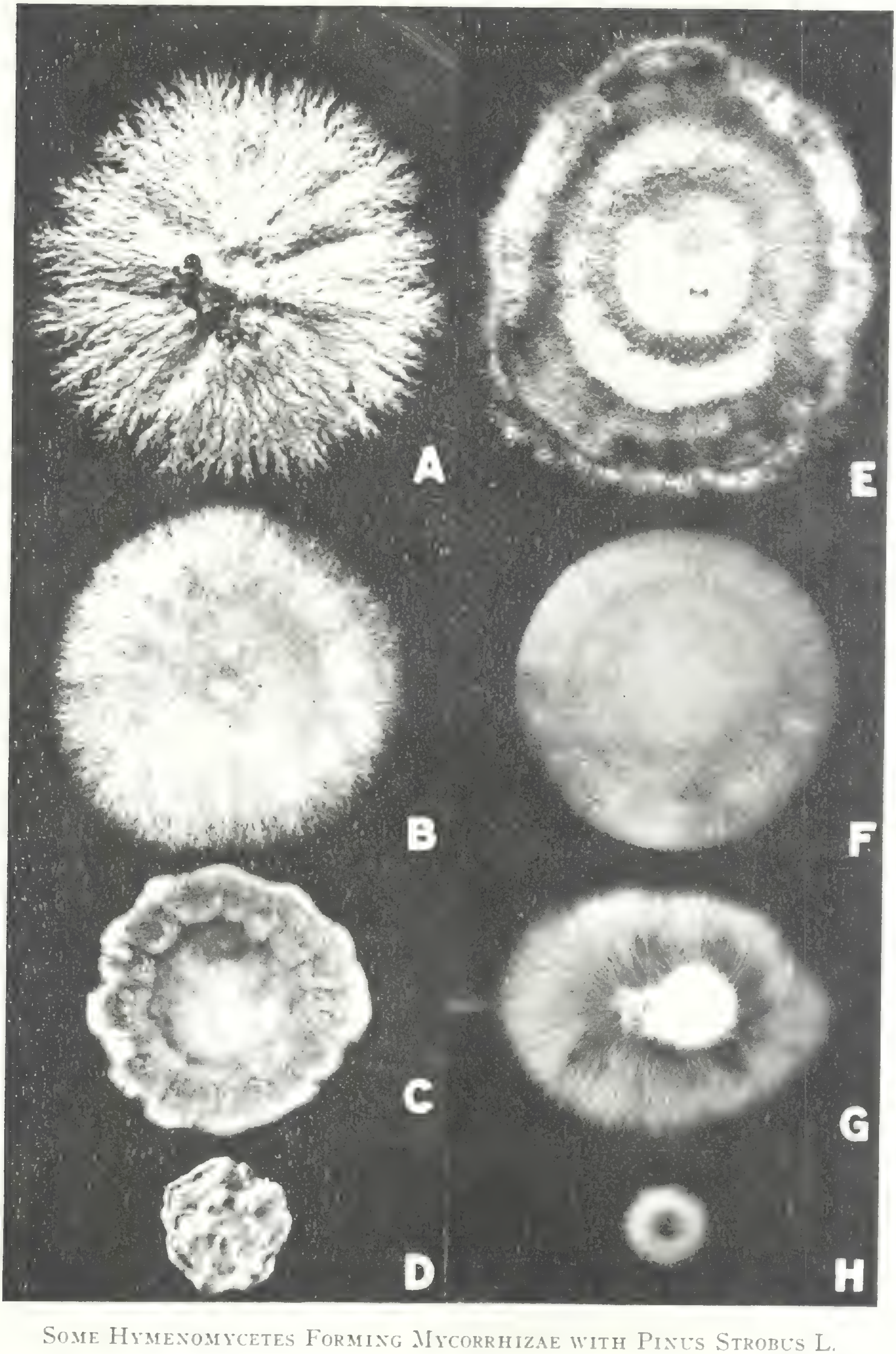
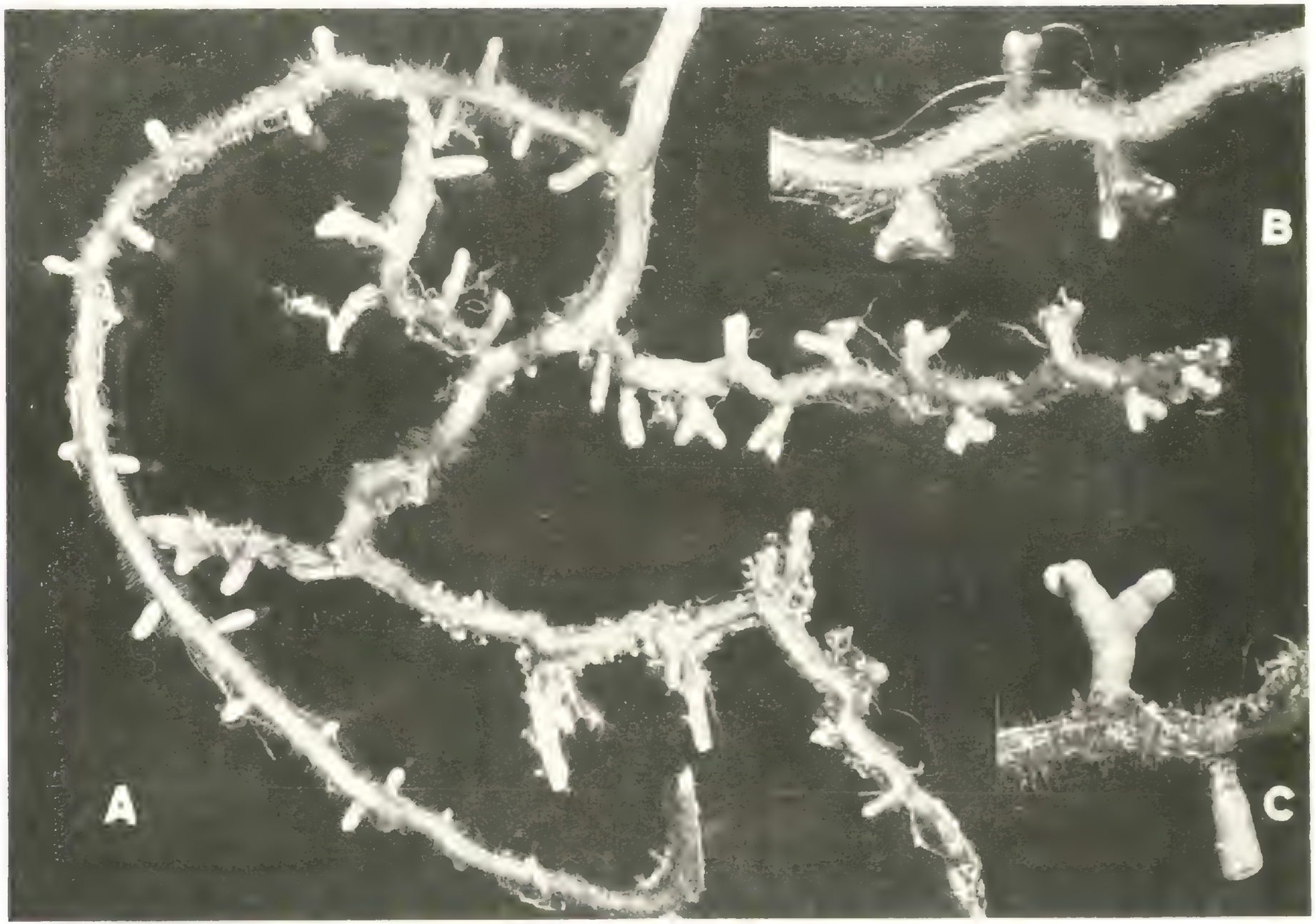
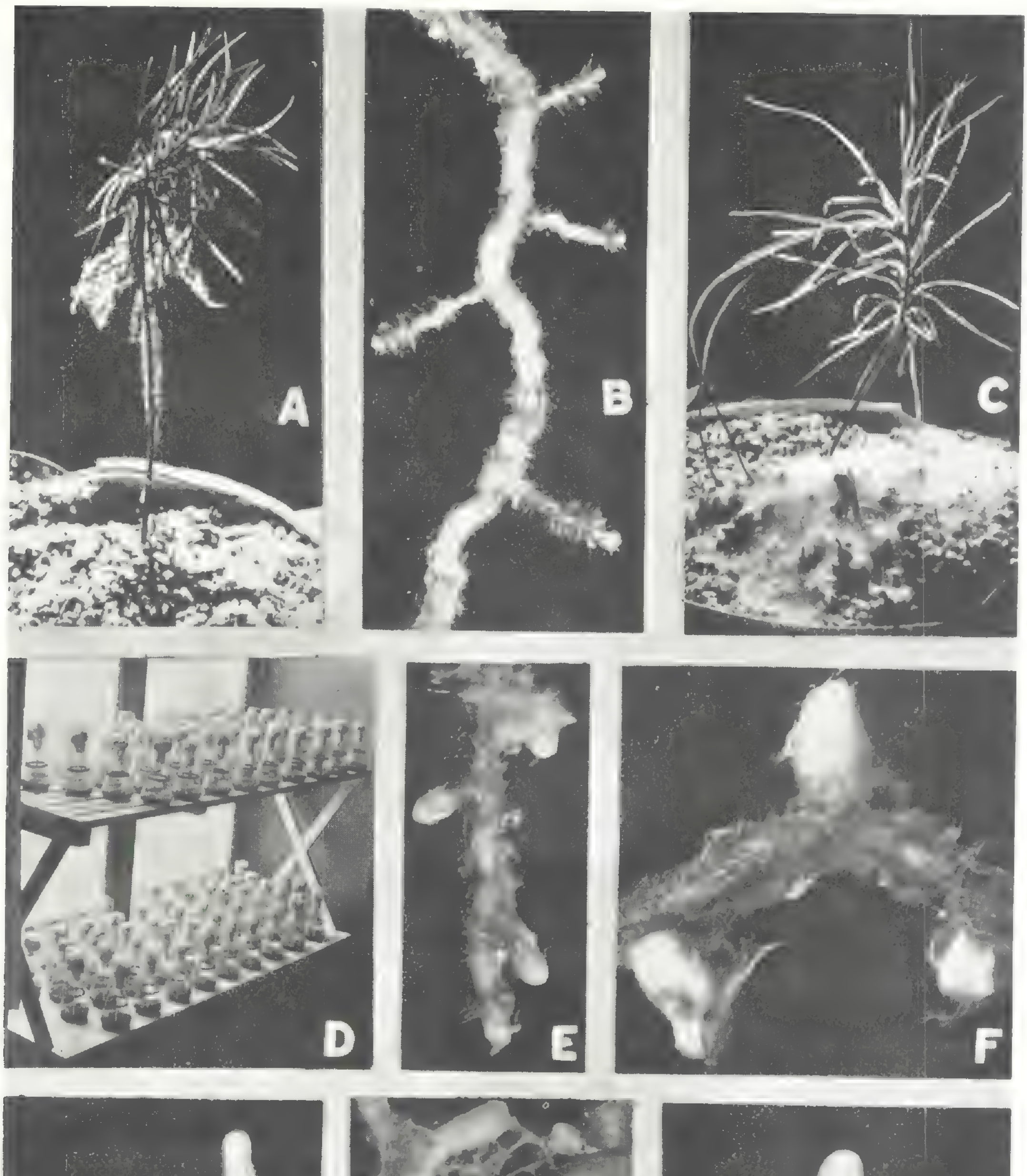
HARVARD UNIVERSITY.



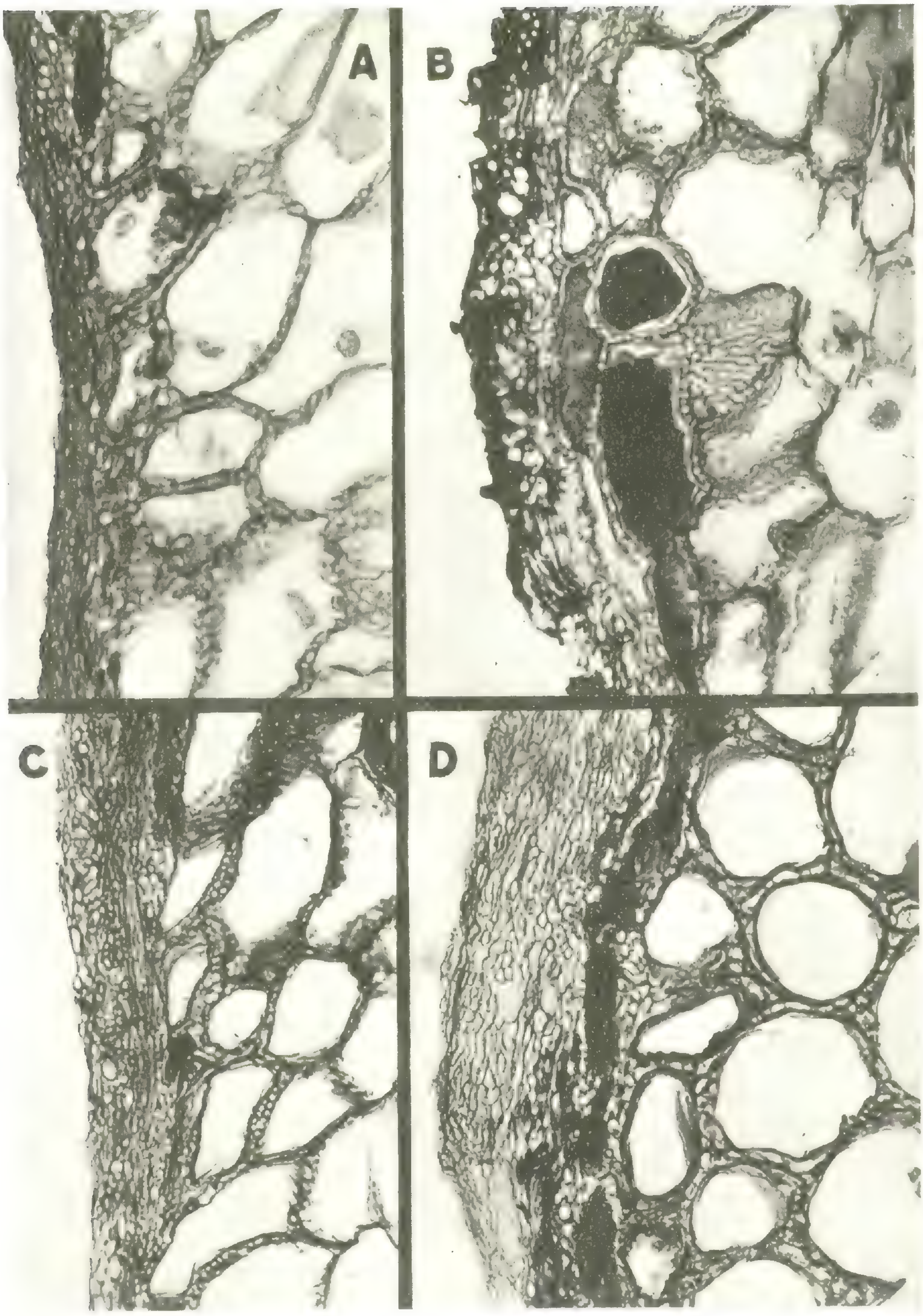
SOME HYMENOMYCETES FORMING MYCORRHIZAE WITH PINUS STROBUS L.



Some Hymenomycetes Forming Mycorrhizae with Pinus Strobus L.



SOME HYMENOMYCETES FORMING MYCORRHIZAE WITH PINUS STROBUS L.


A
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JOURNAL
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NOTES ON THE DISTRIBUTION OF WHITE SPRUCE AND BANKSIAN PINE IN NORTHWESTERN CANADA

HUGH M. RAUP

With plates 72 and 73

THE WHITE SPRUCE, *Picea glauca* Voss (*P. canadensis* of most American authors) is the predominating forest tree throughout the central part of the Mackenzie basin, where its dark green spire-like tops give primary character to nearly every landscape. A traveler descending the Athabaska or Peace River gets the impression that the country is mantled with a heavy spruce forest, but this proves to be an illusion if short inland excursions are made, even if not more than a hundred yards from the river. A considerable part of the timber is found to be on the immediate banks of the rivers, while large areas in the flood plains through which the streams flow are covered with marsh lands separated by low natural levees which hold other narrow bands of timber. This lowland spruce forest is a nearly pure stand of tall, straight-boled trees, with a relatively thick undergrowth of Gray Willow, *Salix Bebbiana,* Mooseberry, *Viburnum pauciflorum,* Red-osier Dogwood, *Cornus stolonifera,* and a wild Rose, *Rosa acicularis.* There is a mat of woodland mosses on the ground in the older of these forests, but it is everywhere thin and in places almost non-existent. A prominent herbaceous species is *Equisetum pratense,* which often makes a continuous greensward on the forest floor. The lowland Spruce is common in suitable situations throughout the central part of the Mackenzie basin and far northward along the main streams. As would be expected it is but scantily developed eastward in the pre-Cambrian country where the thinness of the soils and the absence of large streams has precluded the formation of extensive alluvial deposits.

A spruce timber which is closely related floristically to that of the

lowlands is found on the gently rolling surfaces of the upland country south and west of the Athabaska-Great Slave Lake region where it is interspersed with wide areas of Banksian Pine, *Pinus Banksiana* Lamb. It also occurs, although in a modified form, on the lower slopes of the northern Rocky Mountains in the upper Peace and Liard River basins. Where not disturbed for long periods this forest is a nearly pure stand of the Spruce in the more northern sections, while it is accompanied by *Abies* farther south, *A. lasiocarpa* in the mountains and *A. balsamea* in the southern and southeastern sections. Toward the mountains Banksian Pine is replaced by the Lodge-pole Pine, *P. contorta* var. *latifolia*. Various forms of White Birch, *Betula papyrifera* and var. *occidentalis*, and *B. neoalaskana*, are of frequent though not dominant occurrence. The ground cover is usually a mat of woodland mosses with a very sparse herbaceous growth; and the shrub cover is also thin, consisting chiefly of *Viburnum pauciflorum*, *Alnus crispa*, *Salix Bebbiana*, *Shepherdia canadensis*, and *Rosa acicularis*. The whole differs from that of the lowlands mainly in having a thicker moss cover, relatively lighter shrub growth, and but few herbs. In the mountains the shrubs are more numerous in species and individuals, with the addition of *Acer glabrum* var. *Douglasii* and *Oplopanax horridum*.

The highest and oldest land surfaces on the uplands are on the tops of the erosion plateaus which make the heights of land between the larger streams. The Caribou and Birch Mountains, and the Buffalo Head Hills are typical of these. Their forests have not been extensively investigated, but they seem to be characterized by Cordilleran elements. In the Lesser Slave Lake region they contain the Lodge-pole Pine and the Subalpine Fir, *Abies lasiocarpa*, while at the eastern edge of the Caribou Mountains the former has been found.

Still another type of spruce forest occurs in isolated localities in the central part of the Mackenzie basin as well as in the northern Saskatchewan River drainage of central Alberta. About 20 miles west of the upper Slave River, near the southeastern end of Lane Lake, is a highly morainic country composed largely of sand. On the tops of the highest hills there are open park-like woods in which the individual spruce trees grow with their lower branches on the ground, and in which there is very little shrub growth. The ground cover is principally of lichens, *Cetraria nivalis*, *C. islandica*, *Cladonia alpestris*, etc., and some mat-forming heaths, *Arctostaphylos Uva-ursi*, and *Vaccinium Vitis-Idaea* var. *minus*. Farther westward, near Moose (Eight) Lake, other sandy hills have a similar vegetation. Dr. E. H. Moss has described an iso-

lated stand of Spruce in central Alberta near Edmonton[1] which occupies a similar habitat and seems both floristically and structurally similar to those farther north. It should be noted that these open stands of Spruce occur as "islands" in the ordinary mossy spruce woods described above, or in the dry pine woods. On the high morainic hills, in fact, the Pines occupy the lower slopes while the park-like Spruce is confined to the tops.

Characteristic features of the northwest shores of Lake Athabaska are sand plains and ancient beaches which are many feet above the present level of the lake and form gentle slopes away from the rocky hills. The hills have a scraggly and stunted growth chiefly of scattered Jack Pines, while the plains have an open park-like timber of the same species mixed with Black Spruce, *Picea mariana*, and White Birch.[2] The ground cover in the latter woods is of lichens, *Cladonia alpestris*, *Cetraria nivalis*, etc., and a few mats of *Arctostaphylos Uva-ursi*, *Vaccinium Vitis-Idaea* var. *minus*, and Crowberry, *Empetrum nigrum*. A few taller shrubs are present, the most common being the Blueberry, *Vaccinium canadense*. A further examination of the inland country reveals that the White Spruce may occur in some abundance, but always on local flood plains where small quantities of alluvial silts have been sorted and deposited much as they are along the main rivers. Otherwise it is rather occasional in rock crevices and small soil accumulations in exposed situations. The upland pine woods west of the Slave River grow on sandy soils, and in their younger stages, as will be noted below, closely resemble those just described.

If the observations are continued farther northeastward in the pre-Cambrian country to the eastern part of Great Slave Lake it is noted at once that the Pine has nearly disappeared from the forests, its place being taken almost entirely by the White Spruce. Rocky hills have stunted Spruces with gnarled and twisted trunks, and the sand plains such as are found at the eastern end of the lake have an open spruce wood which has the general aspect of the pine woods farther south.[3] The park-like timber is nearly identical with that of the high morainic hills referred to above.

[1]Moss, E. H. The Vegetation of Alberta, iv. The Poplar Association and Related Vegetation of Central Alberta. (Jour. Ecol. xx. 412-3. 1932.)

[2]Raup, H. M. A Survey of the Vegetation of Shelter Point, Athabaska Lake. (Univ. of Pittsburgh Bull. xxv. Oct. 25, 1928.)

[3]Raup, H. M. The Vegetation of the Fort Reliance Sand Plain. (Annals of the Carnegie Mus. xx. 9-38. 1930.)

To these facts must be added some recent findings on the north shore of Lake Athabaska. About 25 miles northeast of Chipewyan, Sand Point juts out into the lake to a distance of about 2 miles. It is composed entirely of sand, at least at the surface, and has its greatest elevations about a mile from its base. The eastern side of the point has been greatly modified by wind and wave action so that back of a long sand beach there is a steep bank of sliding sand in places about 50 feet high. The tops and upper western slopes of the sandy ridges are more stable and have an open spruce timber similar to that on Great Slave Lake. Other sandy ridges and plains nearer the base of the point are given over to the Pine.

To recapitulate, there are three types of white spruce forests in the central part of the Mackenzie basin. One is of recent development on the flood plains of streams where all the processes in its formation upon newly exposed alluvial deposits may be seen. It is characterized by a rather thin moss cover on the ground, and by a relatively dense undergrowth. A second is widely distributed on the lighter and better drained soils of the uplands, especially where it has not been much disturbed by fire or other agency. It has a much thicker moss mat (4-5 inches) than that of the flood plains, and a rather sparse undergrowth. In the southern sections and in the mountains the Firs are associated with both of these types. The third type has an open, park-like stand of trees in which there is very little undergrowth and no mat of mosses on the ground. The place of the latter is taken by a lichen and heath mat. This type has a scattered distribution in the southwestern portions of the region where it is confined to the tops of high sandy ridges or exposed sandy promontories like Sand Point on Lake Athabaska. On Great Slave Lake, however, it is much more common, covering large areas of sand plain and rocky upland. It is in the form of the last type, with minor modifications, that the Spruce reaches its extreme limit in the arctic tundra.[1]

Random notes made by travelers northeast and east of the Athabaska Lake country suggest a similar arrangement of forest types. J. B. Tyrrell described spruce woods along the Dubaunt and Kazan Rivers which must be like those seen on Great Slave Lake.[1] Also, judging from his descriptions the northern limits of the Jack Pine on

[1]TYRRELL, J. B. Report on the Doobaunt, Kazan and Fergusin Rivers and the North-west Coast of Hudson Bay. (Canad. Geol. Surv. Ann. Rept. IX. 163F and 214F. 1898.)
SETON, E. T. The Arctic Prairies. New York, Charles Scribner's Sons (1911).

these streams are at Selwyn and Theitaga Lakes, respectively, between 50 and 100 miles south of the northern limit of timber. In another report[1] Tyrrell has this interesting observation, "One small isolated grove of White Spruce was found on a high sandy island in Hatchet Lake, standing out conspicuously in the midst of the surrounding forest of small Black Spruce." Hatchet Lake is about 100 miles southeast of the eastern end of Lake Athabaska, and far into the range of the Pine, so that we may look upon this grove as another isolated southern stand of the third type similar to those described above.

There have been striking changes in the land forms of the region in post-Pleistocene time. Topographic evidence indicates that the ice front retreated from southwest to northeast across the country, holding impounded for considerable periods the waters of the Athabaska and Peace Rivers to form a series of post-Glacial lakes which were progressively smaller, lower in elevation, and more recent, to the northeastward.[2] Morainic deposits left by the melting ice included high ridges which must have stood out as islands in the lower lakes. Thus we may establish the soil surfaces of the sandy hill tops west of the Slave River as considerably older than those of their immediate surroundings, and they must have been available for plant cover long before these lower levels. The topographic history of the hill tops noted by Moss in central Alberta has not been studied. With these topographic changes there must have occurred a climate gradually shifting from one similar to that of the present arctic tundra to what we have now. So far as we know there has been no change other than one of amelioration.[3]

The thick spruce woods, then, are on somewhat younger soils of the uplands, and on the still younger silts of the flood plains formed in the last of the lake bottoms. The open spruce woods west of the Slave River are on the oldest hill tops. The Great Slave Lake woods, however, as well as those of Sand Point, though closely related to the ones

[1]Tyrrell, J. B., assisted by Dowling, D. B. Report on the country between Athabaska Lake and Churchill River with notes on two routes traveled between the Churchill and Saskatchewan Rivers. (Canad. Geol. Surv. Ann. Rept. VIII. 12. 1896.)

[2]Cameron, A. E. Post-Glacial Lakes in the Mackenzie River Basin, Northwest Territories, Canada. (Jour. of Geol. XXX. 337-53. 1922.)

Raup, H. M. The distribution and affinities of the vegetation of the Athabaska—Great Slave Lake Region. (Rhodora, XXXII. 187-208. 1930.)

[3]Tyrrell, J. B. Changes of climate in north-western Canada since the glacial period. Die Veränderungen des Klimas seit dem Maximum der Letzten Eiszeit. (11th Internat. Geol. Congress, Stockholm (1910), p. 389-91.)

Raup, H. M. Botanical investigations in the Wood Buffalo Park. In prep.

on the old hills west of the Slave River, are on very recently formed lake shore sand plains and beach ridges. This indicates that the lengths of time since their probable origins will not of itself account for their nature or distribution since they occupy some of the oldest and youngest surfaces

Elevation above the general land surface, with consequent local modification of climatic and edaphic factors may have something to do with the occurrence of this forest on the high ridges west of the Slave River and in central Alberta. But mere elevation appears ineffective since the type also grows very near the level of Lake Athabaska at Sand Point, and on Great Slave Lake. In the latter region the climate in general, as far as it is indicated by the vegetation, is more arctic in character than near the western end of Lake Athabaska, so that we might expect some sort of change in the forests. As noted above, this change is shown by the elimination of the Jack Pine to the northeastward.

The park-like spruce woods at Sand Point and west of the Slave River, although differing widely in the age and elevation of their soils, have this in common—that they are in places which are exposed to cold storm winds, mostly from the north and east, and that their soils are dry and sandy. It is probable that woods of this type in central Alberta may be described in the same way. Similar soils not so exposed have jack pine or mossy spruce woods, but the question of what any other type of soil would produce in such exposed places needs further study. The only evidence we have in this connection is on isolated granite hills in the Athabaska-Peace delta where small patches of clayey soils of early lacustrine origin have natural prairie on them. Undoubtedly a study of the various habitats in the so-called "Barren Lands" or "Arctic Prairies" would throw some light on the matter.

H. E. Pulling, basing his conclusions upon field investigations of roots in north-central Manitoba, suggests that an important factor determining the northern limits of various trees is the relation between the root habits and the permanently frozen condition of the subarctic soils.[1] He classes the Black Spruce, Tamarac, and White Birch as having a rigid shallow root habit, White Spruce as having a flexible shallow root habit, Black Poplar a deep flexible habit, and Jack Pine a deep rigid one. Northward, of course, soils are increasingly shallow due to the frozen subsoil. In general the distribution of trees in the

[1] PULLING, H. E. Root habit and plant distribution in the Far North. (Plant World, XXI. 223-33. 1918.)

central part of the Mackenzie basin may be correlated with these findings. White Spruce, with a shallow flexible root habit, is most successful in coping with subarctic conditions, and extends far out on the Barren Lands in favored places. Other shallow rooted species, but having a rather inflexible system—Birch, Tamarac, and Black Spruce—have a wide range in the Mackenzie basin but are nowhere so tolerant, with the possible exception of the Birch, as the White Spruce. Black Poplar and Jack Pine, with deep root systems, have somewhat similar ranges. The former, like the Pine, has its northeastern limit near the eastern end of Great Slave Lake. Another species, White Pine, also listed by Pulling as having a deep rigid root habit is entirely absent from the region so far as is known.

On the other hand, if this relationship is everywhere of first importance, we should expect to find, for instance, that the sandy soils on Sand Point, on the high moraines west of the Slave River, and on some of the hills in central Alberta, differ from those very near them in being frozen nearer the surface. Although no investigations have been made in these places, it does not seem probable that they would show the sharp differences. Great variations in elevation such as occur between the Slave River and the Caribou Mountains are known to be accompanied by frost differences in the soils, but these are not to be expected, for example, on a low sandy lake shore like Sand Point. Soil frost, therefore, although it is probably of first importance in the general distribution of the species in question, may not account for all the facts relating to the occurrence of the isolated stands of open Spruce; and we must conclude that exposure to storm winds may be a prime factor.

The southern occurrences of northern park-like Spruce may be looked upon as remnants of an earlier forest which occupied the sandy soils throughout the region when a slightly more arctic climate, similar to that now prevailing about the eastern part of Great Slave Lake, was general farther south. There are vast areas of sandy uplands, now covered with jack pine timber, whose soils show no evidence of the humus accumulations which would have arisen from an earlier, heavier timber with a moss mat. These areas must have had an open spruce timber with a thin lichen mat during a somewhat more arctic climate, since the Jack Pine, as indicated by its present range, would then have had its northern limit farther south. These facts suggest that the Jack Pine is the most recent arrival among forest trees in this part of the Mackenzie basin, and that it has replaced the Spruce except in situations excessively exposed.

The mossy spruce woods on the uplands west of the Slave River appear to be developing from jack pine stands by stages which are readily seen. Young groves of the latter are of close-growing, rather spindling trees, but later they thin out and produce much larger crowns with arching branches. During this stage young and vigorous Spruces come up in the shade of the Pines, accompanied by the beginnings of the moss mat. Later stages show a predominating spruce stand in which there are very old, scattered, and dying Pines festooned with lichens, and with small bunches of green needles at the tips of their long branches.

How the Pines invaded the country in the first place, crowding out a park-like spruce forest as suggested above, is uncertain. Study of the country between Athabaska and Great Slave Lakes would undoubtedly reveal a series of intermediate stages which would indicate the transition. It is probable that Pines would invade the open spruce forest in small numbers at first, not enough to make much shade, but sufficient to crowd out the older Spruces and prevent the growth of younger ones. As the climate ameliorated we may suppose that the Pines became more abundant, with such changes in soil and general conditions that Spruces could again invade and form the mossy woods so common now on the more southern uplands. Among the areas thus far studied by the writer those of Lake Athabaska come nearest to illustrating these intermediate stages. As one passes along Sand Point toward the base, the change from open spruce to very open jack pine woods in which many of the trees are old is a gradual one. Still farther inland the Pines are in a closer stand.

The Lake Athabaska pine woods, being farther to the northeastward, nearer the limit of range, and occurring on or near the windswept margins of the lake, are growing under more difficult conditions than those west of the Slave River, and could therefore be considered, as a whole, nearer to an original condition. This is borne out by the above-mentioned gradual transition from old spruce to old pine woods on Sand Point, and by the fact that so far no place has been found where the sand plain pine woods are developing into mossy spruce woods as they are on the uplands. Whether this is due to youth or exposure, or both, is uncertain. They are surely growing upon much younger land surfaces. Furthermore, being near the northeastern limit of its range, the Pine is undoubtedly more selective in its choice of habitats than farther south, and more circumscribed in its ability to invade other associations.

Burning probably aids the spread of pine woods by opening other timber associations, but the normal course of development on the uplands to spruce woods seems to be only retarded by this means. Whether the same agency furthered the replacement of park-like spruce woods by the Pine is uncertain. If it were a very important factor we should expect that the sandy hill top Spruce of the uplands would long ago have been removed. But since it has not we must look for other causes for the present relationships.

SUMMARY

In the distribution of the existing pine and spruce forests of the central part of the Mackenzie basin there is evidence that the earliest post-Glacial timber was one of White Spruce arranged in an open association on sandy soils. The ground cover in this timber was probably a thin mat of lichens and heaths; and the whole persists to the present time as a well-developed type near the arctic timber line. With the amelioration of the climate after the recession of the glacier, other less tolerant species could migrate into the country, among them the Jack Pine. The Pine could successfully compete with the Spruce on the wide-spread sterile sandy soils, and soon formed an open association resembling in aspect that of the Spruce. It is probably typified now by the sand plain pine woods of the Athabaska Lake district.

An increasing growing season, more available moisture, and more rapid decay then permitted the accumulation of organic materials in the soils at a faster rate than had been possible before. This inaugurated a forest succession on the higher and relatively older land surfaces southwest of Great Slave Lake. The richest woods growing in the region now are of White Spruce with a moss mat and light undergrowth; and they appear to develop directly from mature jack pine woods, although in many places burning and the invasion of Trembling Aspen and Black Poplar have prolonged the process. As noted elsewhere by the writer,[1] there is floristic as well as topographic evidence of the relative immaturity of even these richer spruce forests. Many species of wide range across the continent in the coniferous forests are absent or very rare in the Athabaska-Great Slave Lake region, and suggest that the latter have not had sufficient time under the existing subarctic conditions to develop further.

The most recently formed spruce woods are on local flood plain

[1] RAUP, H. M. See p. 339, foot-note 2.

deposits along the present streams. They resemble the upland mossy Spruce but have a thinner ground cover and relatively heavier undergrowth.

In a few local areas far to the south and southwest of the present park-like spruce forest there are woods which appear to be relics of the former wide distribution of this type. They are on both old and very young land surfaces, at both high and low elevations, but are all in places unusually exposed to storm winds. It is supposed that the Jack Pine has not been able to invade the original Spruce in these exposed situations.

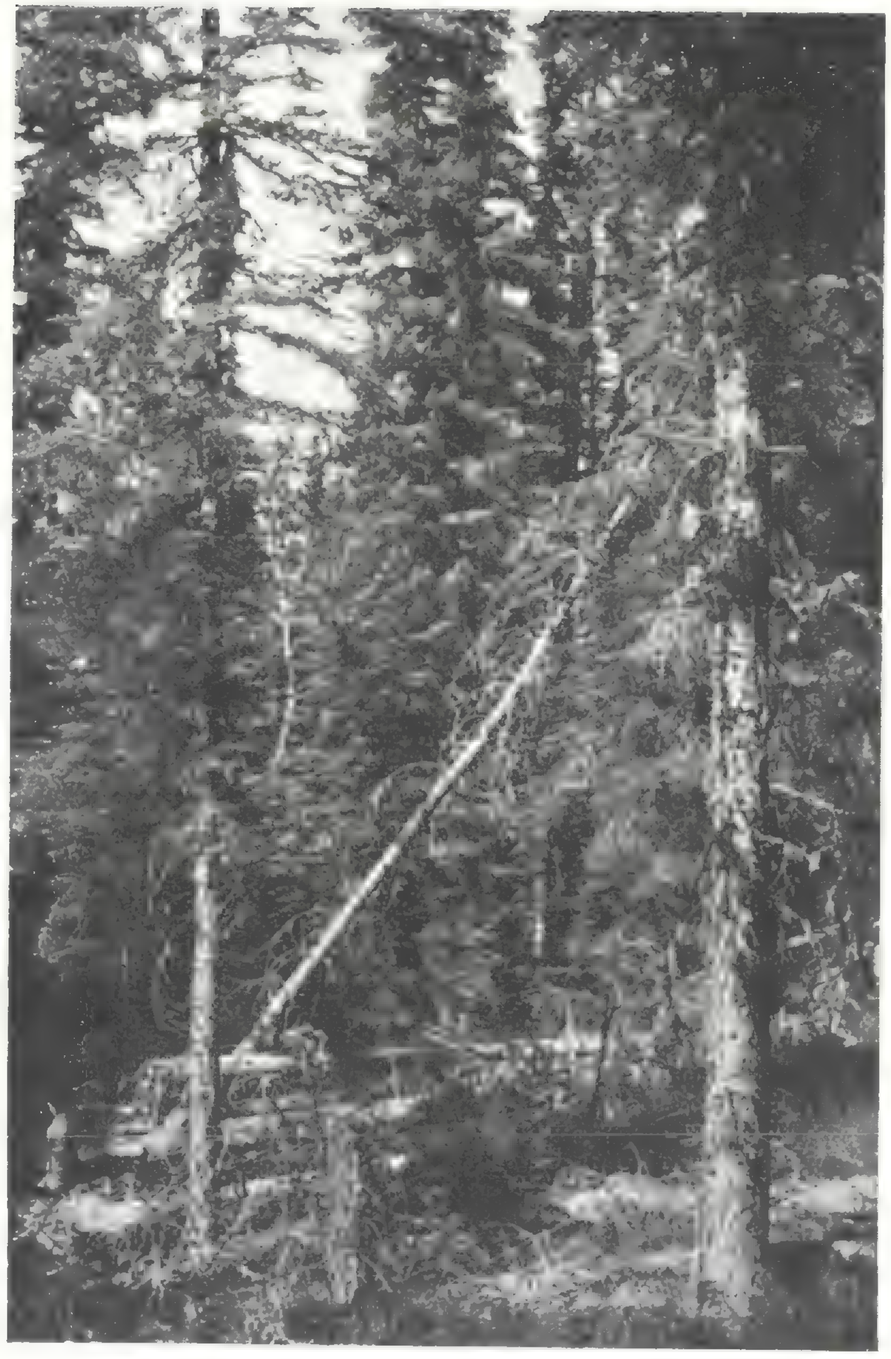
EXPLANATION OF THE PLATES

Plate 72. LEFT: Lowland spruce forest along Peace River near Peace Point. Note ground cover of *Equisetum pratense*.
RIGHT: Upland spruce woods about 40 miles west of the upper Slave River, near Pine Lake.[1]

Plate 73. UPPER: Open spruce woods on sand plain at the eastern end of Great Slave Lake (Ft. Reliance).
LOWER: Open woods of *Pinus Banksiana, Picea mariana,* and *Betula neoalaskana* near Shelter Point, Lake Athabaska.

HERBARIUM, ARNOLD ARBORETUM,
HARVARD UNIVERSITY.

[1]The writer is indebted to the National Museum of Canada for use of the two photographs in this plate.



Distribution of White Spruce and Banksian Pine

Distribution of White Spruce and Banksian Pine

NEW SPECIES, VARIETIES AND COMBINATIONS FROM THE HERBARIUM AND THE COLLECTIONS OF THE ARNOLD ARBORETUM[1]

ALFRED REHDER

With plate 74

Ilex ficoidea Hemsl. in Jour. Linn. Soc. XXIII. 116 (1886). — Loesener in Nov. Act. Leop.-Carol. Akad. Naturf. LXXVIII. 328 (Monog. Aquifol.) (1901).

Symplocos tetramera Rehder in Sargent, Pl. Wilson. II. 598 (1916. — Chung in Mem. Sci. Soc. China, I. 211 (Cat. Trees Shrubs, China) (1924). — **Synon. nov.**

CHINA. Kwangtung: Hongkong, *C. Ford* (? isotype of *I. ficoidea*); same locality, without collector, Feb. 1892; Lantao, Herb. Hongk. no. 8251; Loh fan shan, *C. O. Levine,* nos. 606 and 1567, Oct. 27-30, 1916 and Aug. 15, 1917; Lung tou shan, *S. P. Ko,* no. 50283, Apr. 2, 1930. Fukien: in woods and open thickets, *R. C. Ching,* nos. 2227 and 2231, Aug. 3, 1924. Chekiang: 50 li north of Sia-chu, *R. C. Ching,* no. 1646, May 24, 1924; 130 li of Wen-chou, *R. C. Ching,* nos. 1848 and 1862, June 17, 1924; 80 li northeast of Tai-suan, *R. C. Ching,* no. 2206, July 22, 1924; Hang chow, *T. Tang & W. Y. Hsia,* no. 365, July 18, 1927; Tai-pai-shan, *Y. L. Keng,* no. 1145, Aug. 24, 1927. Anhwei: Wu Yuan, *K. Ling,* no. 7858, Aug. 24, 1924. Hunan: in monte Yun-schan prope urbem Wukang, *Wang-Te-Hui, Handel-Mazzetti,* no. 12810, Apr. 1919; prope urbem Tchang-scha, *Handel-Mazzetti,* no. 11609, Apr. 13, 1918. Yunnan: Puerh, *A. Henry,* no. 13273 (holotype of *Symplocos tetramera*); between Muang hing and Szemao, *J. F. Rock,* no. 2740, March 2-12, 1922.

Ilex ficoidea resembles in its general aspect and particularly in its leaves and in the axillary clusters of its flowers so closely certain species of *Symplocos,* as *S. anomala* Brand, *S. congesta* Benth. and *S. crassifolia* Brand, that also other botanists besides myself had placed in the herbarium specimens of this *Ilex* under the genus *Symplocos.* When

[1]Continued from p. 222.

describing Henry's no. 13273 as *Symplocos tetramera* I explained the different shape of the pistil by the assumption that the ovary was rudimentary and the unusual reduction of the number of stamens by reference to some South American species of *Symplocos* with only four stamens.

Ilex ficoidea has not yet been recorded from western China and in central China only from Changsha, Hunan (Handel-Mazzetti, no. 11609) and from Wukang, Hunan (Handel-Mazzetti, no. 12810, as var. *brachyphylla* Hand.-Mazz.). Henry's no. 13273 differs somewhat from typical *I. ficoidea* in the shorter and stouter petiole, about 5 mm. long, and in the shorter acumen of the leaves. Rock's no. 2740 which also has been distributed as *Symplocos tetramera,* is similar but the leaves are comparatively narrower and generally oblong and of thinner texture.

Acer sikkimense Miq. var. **serrulatum** Pax in Bot. Jahrb. VII. 215 (1886); in Engler, Pflanzenr. IV-163, p. 34 (1902). — Rehder in Sargent, Trees & Shrubs, I. 180 (1905). — Fang in Contrib. Biol. Lab. Sci. Soc. China, VIII. 178 (1932).

CHINA. Yunnan: Feng-cheng-lin Mt., forests, south of Red River, alt. 7000 ft., *A. Henry,* no. 10640 (tree 10 ft.); west of Talifu, Mekong watershed, en route to Youngchang and Tengyueh, *J. F. Rock,* no. 6834, Sept.-Oct. 1922; Shweli river drainage basin, environs of Tengyueh, *J. F. Rock,* no. 8014, Feb. 1923; without precise locality, *G. Forrest,* nos. 9759 and 26233.

HIMALAYA: Bengal, *Griffith,* no. 936 (holotype; not seen).

Acer sikkimense agrees with *A. Davidi* in having oblong-ovate leaves without lobes, but differs in their caudate-acuminate apex, subcordate base, glabrous under side, entire or nearly entire margin and in the shorter pedicels and longer racemes. In the variety the leaves are closely and sharply serrulate.

In my account of the Maples of the sect. Macrantha (pp. 211-222) I unfortunately overlooked this Chinese variety of an Himalayan species. In the key it should precede *A. Davidi* from which it is easily distinguished by the closely and finely serrulate margin of the leaves and their glabrous under side which in *A. Davidii* is more or less rufous-tomentose along the veins at least when young.

Acer Davidi Franchet in Nouv. Arch. Mus. Paris, ser. 2, VIII. 212 (Pl. David. II. 30) (1884). — Fang in Contrib. Biol. Lab. Sci. Soc.

China, VIII. 177 (1932). — Rehder in Jour. Arnold Arb. XIV. 213 (1933). — Add the following synonym:

Acer laxiflorum Pax var. *β ningpoense* Pax in Engler, Pflanzenr. IV.-163, p. 36 (1902). — Rehder in Sargent, Trees & Shrubs, I. 180 (1905).

CHINA. Chekiang: Ningpo-Berge, *E. Faber*, in 1886 (holotype in Herb. Berol.; photo. and fragments in A. A.).

In my account of the Chinese Maples of the Macrantha section in the last number of this Journal I had omitted *A. laxiflorum* var. *ningpoense*, because I had not seen the type and doubted very much the possibility that a variety of the western *A. laxiflorum* should occur near Ningpo. I have now received through the kindness of Professor R. Pilger of the Botanical Museum at Berlin-Dahlem an excellent photograph and fragments of the type specimen which proves that the specimen does not belong to *A. laxiflorum* but is a slight variation of *A. Davidi* with indistinctly lobulate leaves and horizontal wings of the fruit. It differs from *A. laxiflorum* in the ovate-oblong leaves not 3—5-nerved at the base and abruptly narrowed into a remotely serrulate acumen entire toward the tip, in the irregular crenate-dentate serration, and the practically glabrous under side, while in typical *A. laxiflorum* the leaves are sharply serrulate with acuminulate teeth, distinctly 3-lobed and 3—5-nerved at the very base, with an elongated triangular-ovate middle lobe gradually narrowed in a closely and sharply serrulate caudate acumen, and are even at maturity more or less rusty pubescent on the veins. From slightly lobed forms of *A. Grosseri* Pax Faber's Ningpo specimen differs chiefly in the oblong or oblong-ovate shape of the leaf, its crenate-dentate serration and the not clearly 3-nerved base.

Rhamnus crenata S. & Z. var. **discolor,** var. nov.

A typo recedit foliis subtus albido-tomentosis, nervis utrinsecus 8-12. — Frutex; folia elliptica vel obovato-oblonga ad oblongo-oblanceolata, subito acuminata, basi late vel sensim cuneata, circiter 8 × 4 to 10 × 3.

CHINA. Chekiang: open thickets, alt. 4400 ft., *R. C. Ching*, no. 2536 (type), Aug. 31, 1924 (shrub 15 ft.); southern Chekiang, open thickets, alt. 4200 ft., *R. C. Ching*, no. 2461, Aug. 24, 1924 (shrub 8 ft.).

On account of the whitish tomentose under side of the leaves this variety looks quite different from the type, but I can find no other characters to separate it from *R. crenata* which shows considerable

variation in the shape and also in the pubescence of its leaves, except that the leaves have about 8—12 pairs of lateral veins while in typical *R. crenata* they have only 5—9 pairs.

The two specimens cited above differ considerably in the shape of the leaves: no. 2536 has generally elliptic leaves about 8 cm. long and 4 cm. broad, broad cuneate at base, while no. 2461 has narrow-oblong to oblong-oblanceolate leaves 8—10 cm. long and 2.5—3 cm. broad, cuneate at base, but one leaf is oblong-obovate 9 cm. long and 4.5 cm. broad.

Rhamnus utilis Dcne. var. **hypochrysa** (Schneid.), var. nov.

Rhamnus crenatus E. Pritzel in Bot. Jahrb. XXIX. 460 (1900), pro parte. — Non Sieb. & Zucc.

Rhamnus hypochrysus C. K. Schneider in Notizbl. Bot. Gart. Mus. Berlin, v. 76 (1908); Ill. Laubholzk. II. 290, fig. 198 p-q, 199 o-p (1909); in Sargent, Pl. Wilson. II. 252 (1914).

CHINA. Szechuan: Nanchuan, *A. von Rosthorn,* no. 1585 (syntype of *R. hypochrysus* in Herb. Berlin); Nanchuan Hsien, *W. P. Fang,* no. 1415, June 6, 1926. Shensi: Lin-hua-zao near Kin-lin-san, *G. Giraldi,* nos. 931, 932; Fu-kio, *G. Giraldi,* no. 940 (syntypes of *R. hypochrysus* in Herb. Berlin). Northern Honan: Yung-ning, Tsi-li-ping, alt. 1000 m., *J. Hers,* no. 1368, Sept. 30, 1919; Yung-ning, Yo-tze-ping, alt. 900 m., *J. Hers,* no. 820, Oct. 4, 1919. Hopei: Tang-san, *F. N. Meyer* (seeds only); plants raised from these seeds distributed as U. S. Dept. Agr. no. 17909 in Arnold Arb. coll. 1909 and 1914 (in part). Fukien: Enghok Hsien, *H. H. Chung,* no. 1342, April 14, 1923; Diongloh, Ne-lan-san, Herb. Fukien Christ. Coll. no. 11729, Aug. 6, 1926.

This variety differs from typical *R. utilis* chiefly in the pubescent branchlets and pubescent leaves. In the type specimens of *R. hypochrysus* and in Fang's no. 1415 the pubescence is rather dense and distinctly yellow, while in the specimens from Honan the pubescence is somewhat slighter and not or scarcely yellow. In the Fukien specimens which have rather small leaves, the yellowish pubescence is still slighter and the branchlets are only very sparingly pilose or nearly glabrous, so that these specimens closely connect typical *R. utilis* with the variety. From the seed collected by F. N. Meyer near Tang-san, which is apparently the same as Tang-shan between Tien-tsin and Yung-ping-fu, and distributed from the U. S. Department of Agriculture under no. 17909, both forms, the glabrous and the pubescent with distinctly villous branchlets, were raised. It, therefore, does not seem possible to maintain *R. hypochrysa* as a distinct species.

Though Decaisne describes his species as with "foliis . . . subtus puberulis," almost all the numerous specimens in this herbarium except those cited above, have the leaves nearly or quite glabrous. A specimen, however, collected by C. Schneider in 1903 in the nursery of Simon-Louis at Plantières near Metz from a plant marked "R. utilis, original from Decaisne" has the leaves loosely and yellowish pubescent on the under side and when young also slightly so above, but the branchlets are glabrous; this agrees exactly with Decaisne's description.

It, therefore, appears that the type of *R. utilis* is the form with only slightly pubescent leaves but glabrous branchlets, and that the form with the leaves quite glabrous or slightly pubescent only on the veins beneath, may be considered a distinct form and distinguished as **R. utilis** f. **glabra,** forma nova, of which E. H. Wilson, no. 622, from Fang Hsien, Hupeh, may be designated as the type.

Ampelopsis Delavayana Planchon in De Candolle Monog. Phaner. v. 458 (1887).

Amelopsis aconitifolia Bge. γ *tomentella* Diels & Gilg in Bot. Jahrb. XXIX. 465 (1900). — **Synon. nov.**

CHINA. Szechuan: Nan-chuan, *Bock & von Rosthorn,* no. 1540 (holotype of *A. aconitifolia* var. *tomentella;* photo. in A. A.; flowering branch).

Ampelopsis aconitifolia f. *tomentella* the type of which I have seen in the herbarium of the Botanic Museum at Oslo, though superficially quite similar to *A. aconitifolia* f. *setulosa* Diels & Gilg, agrees with *A. Delavayana* Planch. in the occasional presence of undivided only slightly lobed leaves, the shallow serration of the leaflets and the rather short peduncles about as long as the cyme itself. *Ampelopsis aconitifolia* Bge. never has undivided leaves, the leaflets are rather deeply serrate to pinnatifid and the loose and smaller cymes are borne on slender peduncles longer than the cyme. In fruit the two species differ considerably in the color of the berries; the pubescence varies in the two species and glabrous and pilose forms occur in both.

The other forms described (l. c.) by Diels and Gilg: f. *glabra,* f. *setulosa* (pr. parte typ.) and f. *cuneata* belong without doubt to the variable *A. aconitifolia.*

Wilson's specimen no. 1070 (Veitch Exped.) placed in the Berlin Herbarium with *Ampelopsis aconitifolia* f. *tomentella* belongs to *Vitis Piasezkii* Maxim. To *V. Piasezkii* belongs also Henry, no. 6479, one of the syntypes of *Ampelopsis aconitifolia* f. *setulosa.*

Agapetes stenantha, spec. nov. Plate 74

Frutex vel arbor parva, glabra, ramis cinereo-fuscis lenticellatis. Folia coriacea, lanceolata, 6.5—9 cm. longa et 2—2.4 cm. lata, longe acuminata, basi in petiolum brevissimum 1—2 mm. longum attenuata, integra, supra laete viridia, subtus pallidiora, costa media utrinque elevata, nervis utrinsecus 6-7 supra fere obsoletis subtus in sicco levissime elevatis. Racemi axillares e gemmis nudis in ramulis biennibus vel vetustioribus, 5—8 cm. longi, multiflori (floribus circiter 18—25); Flores rubescentes, graciliter pedicellati pedicellis 5—8 mm. longis, bractea lanceolata circ. 2 mm. longa rubescente membranacea decidua suffultis, basi bracteolis 2 bracteae similibus sed saepius paullo minoribus munitis, apice articulatis; dentes calycinis triangulari-lanceolati, circ. 3 mm. longi, dorso costati costis in tubum ovarii fere aequilongum decurrentibus; corolla anguste cylindrica apicem versus leviter angustata, circ. 2 cm. longa et medio 3 mm. lata, subalato-costata, lobis linearibus 1 mm. latis apice obtusiusculo mucrone reflexo munitis, valde inaequalibus, sinu altissimo circiter mediam corollam attingente; stamina stylo paullo breviora supérne in tubum stylum arcte cingentem cohaerentia, antheris pilosulis 5 mm. longis apice in tubum gracilem circ. 1 cm. longum attenuatis, basi in calcar pilosulum, 1.5 mm. longum filamentum glabrum fere aequans productis; stylus glaber, corollam aequans, apice clavatus; ovarium 2-2.5 cm. longum, glabrum.

BURMA: between Sadon and the Yunnan Chinese border at Changtifang and Kambaiti, alt. 7600 ft., *J. F. Rock,* no. 7514, Nov. 1922 (shrub or small tree, flowers bright reddish, very ornamental).

This distinct new species is characterized by the very narrow tubular flowers about 2 cm. long and only 3 mm. wide, borne in many-flowered elongated racemes and by the lanceolate long-acuminate leaves attenuate at base and 6.5—9 cm. long. It differs in the elongated many-flowered racemes from all Asiatic species except *A. vaccinioides* Dunn which is easily distinguished by the short campanulate corolla and the larger leaves rounded or subcordate at base, and except *A. corallina* Cowan which differs in the lanceolate corolla-lobes and apparently glabrous anthers without spur.

(*To be continued*)

HERBARIUM, ARNOLD ARBORETUM,
HARVARD UNIVERSITY.

AGAPETES STENANTHA Rehd.

THE COMPARATIVE ANATOMY OF THE STEMS OF BETULA PUMILA, BETULA LENTA, AND THE HYBRID BETULA JACKII

SARAH M. COUSINS

PROFESSOR JACK(1), in 1895, described a hybrid *Betula,* afterwards named *B. Jackii* by C. Schneider, six individuals of which appeared in a group of several hundred seedlings grown at the Arnold Arboretum. The seed from which the plants were grown was collected from a plant of *B. pumila,* the Dwarf Birch, growing about one hundred paces east of several trees of *B. lenta,* which was the only other species growing in the vicinity, whose pollen would be carried to it by the winds. Jack described the characters of the hybrids and showed that of the six plants four were morphologically intermediate in all ways between *B. pumila* and *B. lenta* and had the aromatic smell characteristic of *B. lenta,* though to a lesser degree. The other two plants were more like *B. pumila* than *B. lenta,* and had little or no aromatic odor.

In 1929 Professor Woodworth(2) described his cytological investigation on the reduction divisions of *B. pumila, B. lenta* and *B. Jackii* in material collected at the Arnold Arboretum, the results of which substantiate Professor Jack's conclusion that *B. Jackii* is a hybrid between *B. pumila* and *B. lenta.* Professor Woodworth said that "Meiosis and pollen formation are typically normal in both the parents," but that *B. Jackii* displays to a marked degree the meiotic irregularities characteristic of hybrids—abnormality of gemini formation, lagging of chromosomes, extrusion of chromosomes into cytoplasm, polyspory, and partial sterility of pollen; he concluded that *B. Jackii* is a hybrid between the diploid *B. lenta* and the tetraploid *B. pumila.*

In view of these investigations it seemed worth while to study the stem anatomy of the three species.

MATERIAL AND METHODS

Material was collected in 1928 from labeled plants at the Arnold Arboretum, killed in Carnoy's, imbedded, sectioned, stained with Heidenhain's iron hematoxylin and safranin, and studied. To check the results obtained, material was again collected in 1933 at the Arboretum, but from individual plants other than those from which the first collections were made, sectioned and stained without imbedding, and

studied. The ages, according to annual ring count, of the oldest specimens studied for each species are:

B. pumila	24 years
B. lenta	18 years
B. Jackii	14 years

The plants of *B. Jackii* were morphologically intermediate between the parents.

COMPARATIVE ANATOMY

All three Birches have a typical woody siphonostele. The differences in anatomy which seem significant are as follows:

PHLOEM SCLERENCHYMA

In all three Birches, groups of phloëm cells become sclerenchymatized, but the locations of the sclerenchymatous tissues in them differ as follows:

B. pumila: phloëm cells that are to become sclerenchyma enlarge and their walls thicken soon after they are formed, consequently the sclerenchymatous bands almost touch the cambium, and depress it. This depression of the cambium under the sclerenchyma masses results in wavy or scalloped annual rings, a characteristic feature after the first year or two of growth. The sclerenchyma masses show a tendency to be continuous radially, that is, to appear as radial bands in transverse section.

B. lenta: walls of phloëm cells that are to become sclerenchyma do not thicken until several years after the cells have formed; consequently there is no sclerenchyma near the cambium, the cambium is not pressed inward, and the annual rings are not scalloped. The sclerenchyma masses do not show any tendency to be continuous radially (but sometimes a slight tendency to be continuous tangentially); they appear in transverse section as small, more or less isodiametric areas.

B. Jackii: as in *B. lenta*, walls of phloëm cells that are to become sclerenchyma do not thicken until several years after the cells have formed, so there is no sclerenchyma near the cambium, and no depression of the cambium or scalloping of the annual rings. The sclerenchyma masses appear in transverse section as small, more or less isodiametric areas; but they differ perhaps from those of *B. lenta* in that they seem sometimes to be nearer the cambium, and show practically no tendency to merge tangentially.

"Aggregate ray" areas

B. pumila: the wood formed by the depressed cambium, under the sclerenchyma masses, has an aggregate ray-like appearance in that it lacks vessels and is composed wholly of rays and fibers; the fibers, especially in older wood, differ from the usual fibers near vessels in the following respects: they are greater in diameter, they are one-fourth to one-third the length of the usual fiber, their ends are oblique or bluntly tapering instead of long and tapering, and they tend to twist and intertwine instead of being comparatively straight up and down. These areas are usually apparent in transverse section in the second or third annual ring, and broaden as they extend out to the cambium.

B. lenta and *B. Jackii*: no such "aggregate ray" area was apparent in any of the specimens of the pollen parent or the hybrid that were examined.

Vessels

The differences between the vessels of the three Birches are small; perhaps if a larger group of specimens was studied they would be found not valid, but in the specimens studied these generalizations seem to hold:

B. pumila: vessels are more or less in radial rows, rather angular, small, and somewhat more concentrated in the spring wood.

B. lenta: except in the first ring, where the vessels resemble those of *B. pumila* in size and distribution, the vessels are in rounded groups of one to about four, or less frequently in radial rows; the groups are scattered evenly throughout the annual ring; the vessels themselves are large and rounded. In radial sections the wood of *B. lenta* can be distinguished from that of the other two types by the fact that the ends of the larger vessels have the bars of the scalariform perforation fewer and further apart than do those of either of the other two forms; the bars are about 12 microns apart in *B. lenta*, and 3.8 to 6 microns in *B. pumila* and the hybrid.

B. Jackii: vessels are in rounded groups or radial rows and are perhaps slightly more concentrated in the spring wood. The general appearance of the transverse section is more like that of *B. lenta* but differs from it in that the groups of vessels seem fewer and further apart and the vessels themselves perhaps smaller.

Rays

All three Birches have simple rays and small compound rays; the

width in cells of the largest of the compound rays seems to be a specific character. The walls of the cells seem to vary in thickness and regularity in the same way in which the walls of the pith cells vary (see below), but to a lesser degree.

B. pumila: the rays are mostly simple, one cell wide, some are compound, two cells wide, and in the "aggregate ray" area some individual rays appear in tangential section to be three cells wide, although this appearance may be due to twisting of cells in the area. In general, however, the rays are not more than two cells wide even in the twenty-fourth annual ring. The walls of the ray cells are more or less irregular, but much less definitely and constantly so than the walls of the pith cells.

B. lenta: rays are from one to three cells wide even in the earlier rings (about the second to sixth); in older wood (about the fifteenth ring), they may be four cells wide. The walls of the ray cells are thickened fairly evenly, but not as evenly as those of the pith cells.

B. Jackii: rays are one, two, and occasionally three cells wide in the earlier rings, often three cells wide in the later ones (about the twelfth). In tangential section *B. Jackii* resembles *B. lenta* more than *B. pumila*. The cell walls are thickened somewhat irregularly; but in all three kinds the characters of the ray cell walls are too similar and inconstant to be of value in distinguishing the species.

PITH

The pith cells of the two parent species present quite distinct and different characters, and those of the hybrid seem intermediate between them. The distinguishing wall characters are seen best in transverse section.

B. pumila: the secondary thickening of the wall is irregular, so that the wall extends into the cell lumen as blunt projections between which are canals of varying diameter ending in simple pits. The thickness of the wall, from the lamella to the inner points of the projections, is usually 7 to 10 microns, about four times that of the wall of the pith cells of *B. lenta*. In longitudinal section the cells are usually twice as long as wide, or longer.

B. lenta: the pith cell wall is thickened evenly so that its inner surface is parallel throughout with its outer one; it is 2 to 3 microns thick and is pierced by pits of constant diameter. The cells are usually less than twice as long as wide (often they are isodiametric), although exceptions occur, especially near the outside edges of the pith.

B. Jackii: the pith cell wall is slightly irregular and about 2 to 5 microns thick; the diameter of its pits vary somewhat. The cells range from isodiametric to more than twice as long as wide. In general the pith cells of the hybrid seem to be intermediate between those of the two parents.

SUMMARY

Betula pumila and *Betula lenta* have several distinct anatomical characteristics; in some of them the hybrid, *Betula Jackii*, seems more or less intermediate—size and distribution of vessels, width in cells of compound rays, proportions and wall characteristics of pith cells.

In other respects the hybrid resembles *B. lenta*, its pollen parent, since they both lack completely in the specimens studied the "aggregate ray" areas, the scalloped cambium and annual rings, and the early-forming phloëm sclerenchyma that characterize *B. pumila*.

In the character of the perforations of the vessel ends the hybrid is unlike *B. lenta*, and closely resembles *B. pumila*.

The writer wishes to acknowledge the kindly suggestions and assistance of Professor R. H. Woodworth, under whose direction this work was done.

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CHROMOSOME NUMBER AND MORPHOLOGY IN THE CONIFERS

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With plates 75-79 and three text figures

THE EXISTING CONIFERS are undoubtedly members of a very old group of plants. Although they constitute the predominant flora of the temperate zone, the number of genera is less than fifty, and many of these are of restricted distribution, and some are monotypic. As a whole, the genera are well differentiated on the basis of floral, anatomical, and gametophytic characters. Species characters are usually well marked and are frequently associated with geographic distribution. The stability of the Conifers would seem to indicate that evolution in this group of plants has passed its climax and that the existing forms are survivors of long natural selection.

A study of chromosome morphology in the Conifers should be of interest in relation to the distinct and long established differentiation of different genera. Previous cytological work shows that the basic chromosome number is usually 12 in the Conifers, and in all other Gymnosperms, with the exception of the Gnetales. Many of these counts were made incidental to morphological studies. A critical study of chromosome morphology has been made in only a few species.

The taxonomic grouping of the Conifers followed in this paper is based on Rehder's (1927) Manual. The Conifers are taken to include the Taxaceae and the Pinaceae. The Pinaceae are divided into four sub-families, the Cupressineae, Taxodineae, Araucarineae, and Abietineae. Representatives of all groups have been examined, with the exception of the Araucarineae. The analysis of the somatic chromosomes was done by the senior author, and the meiotic chromosomes were studied by the junior author.

MORPHOLOGY OF SOMATIC CHROMOSOMES

Preparations of root tips of Conifers are not suitable for a study of somatic chromosomes. The chromosomes are long, and the arms are usually oriented more or less at right angles to the metaphase plate so that polar views of division figures offer little opportunity for a comparison of individual chromosomes, and the numbers are too large to

permit an adequate study from side views of division figures. The somatic chromosomes are best observed in the early development of the endosperm. The chromosomes in this tissue are haploid in number, divisions are numerous, and the chromosomes can be studied from either polar or side views when flattened in aceto-carmine smears.

The entire endosperms were dissected out of the female cones and fixed in absolute alcohol-acetic acid, 70 parts of alcohol to 30 parts acetic. Aceto-carmine smears were made after the material had been fixed for several hours or longer. In some cases the endosperms were changed to 80 percent alcohol after fixing for 24 hours in the alcohol-acetic solution. Aceto-carmine smears can be obtained from such material at any time.

The endosperms were smeared in aceto-carmine containing enough haematoxylin and iron alum to produce a sharp stain. After the slide had been covered, the preparation was heated, and the cover pressed firmly with filter paper to flatten the cells and remove excess aceto-carmine. When sealed, such preparations remain in good condition for several weeks.

The various stages of mitosis in the endosperm of *Cephalotaxus* are shown in plate 75 in order to illustrate the types of figures obtained. The photographs are from preparations which had been flattened sufficiently to include all chromosomes of a given cell in approximately the same focus. During the prophase stage, a single large nucleolus is always found (Fig. 1). At metaphase the chromosomes are U-shaped, with the spindle fiber attachment points in approximately the same plane (Fig. 2). The chromosomes begin to separate at the fiber attachment point, and usually the opening at this region, or the protrusion of attachment points, can be observed at metaphase. The actual separation of daughter chromosomes proceeds rapidly, although occasional figures are found where the chromosomes are still associated at the distal ends (Fig. 3). At late anaphase the individual chromosomes are clearly defined, and the relative lengths of the arms are easily determined (Fig. 4). In *Cephalotaxus Fortuni* 11 chromosomes are approximately isobrachial and one is heterobrachial with a trabant at the end of the short arm. At telophase the chromosomes form a compact mass of chromatin (Fig. 5), and as the daughter nuclei are reorganized before passing into the resting stage, the chromosomes appear to be polarized and to surround a single large nucleolus (Fig. 6). The chromosomes vary considerably in size, even in the same endosperm. They seem to be larger in the free nuclear divisions and become

smaller when cell walls are formed. *Cephalotaxus* seems to have larger chromosomes than the other Conifers studied, but little or no consistent difference is found in the size of chromosomes of other genera.

The chromosomes of *Ginkgo biloba* were included in this study because this species is the only surviving member of the Ginkgoaceae. The haploid chromosome complement from endosperm tissue is represented in figure 7 (Pl. 76). Two of the 12 chromosomes are approximately isobrachial, while 10 of them have sub-terminal fiber attachment points.

The only available genera for study in Taxaceae were *Cephalotaxus* and *Taxus*. *Torreya* is represented in the Arnold Arboretum, but female cones are seldom produced. Twelve chromosomes were found in each of the three species of *Taxus* examined, *T. canadensis, T. baccata,* and *T. cuspidata* (Pl. 76, figs. 8, 9, and 10). In each case one chromosome has a terminal or subterminal attachment point, one is distinctly heterobrachial, while the others are more or less isobrachial.

In the Pinaceae, the sub-family Abietineae has been studied most extensively. The chromosomes of *Pinus* are especially clearly defined. Twelve chromosomes have been found in *P. Banksiana, P. Bungeana, P. flexilis, P. Jeffreyi, P. mugo rotundata, P. nigra, P. parviflora, P. peuce, P. ponderosa, P. resinosa, P. Strobus, P. sylvestris, P. Thunbergiana,* and *P. virginiana*. The chromosomes of all species of *Pinus* seem to be very similar. One of the 12 chromosomes is somewhat heterobrachial, and the others have approximately median fiber attachments. The chromosomes of three species, *P. parviflora, P. Thunbergiana,* and *P. ponderosa,* are illustrated (Pl. 76, figs. 11, 12 and 13).

The chromosomes of *Cedrus libanotica* are morphologically much like those of *Pinus* (Pl. 76, fig. 14). One of the 12 chromosomes is distinctly heterobrachial, and the others are approximately isobrachial.

Two species of *Larix* have been examined for chromosome morphology. The chromosomes of *L. Kaempferi* are shown at late anaphase in figure 15 (Pl. 76), and those of *L. decidua* at metaphase are shown in figure 16 (Pl. 76). The chromosomes of these two species are very similar; 6 of the 12 chromosomes are distinctly heterobrachial, and 6 have approximately median fiber attachment points.

The chromosomes of *Pseudolarix* are unusual among the Conifers, both in number and morphology. There are 22 chromosomes, 20 with terminal or subterminal fiber attachments, and two with more nearly median attachments, although both are distinctly heterobrachial (Pl.

76, fig. 17). The same condition was found in three different trees of *Pseudolarix amabilis*.

Twelve chromosomes have been found in all species of *Picea* examined. The chromosomes of *P. pungens* and *P. Abies* are very similar (Pl. 76, fig. 18 and Pl. 77, fig. 19). Three of the chromosomes are clearly heterobrachial, while the others are more or less isobrachial.

The chromosomes of *Tsuga canadensis* (Pl. 77, fig. 20) and *T. caroliniana* are very similar and resemble those of *Picea*. Three of the 12 chromosomes are distinctly heterobrachial, and 9 have approximately median fiber constrictions. One of the heterobrachial chromosomes has a secondary constriction.

Pseudotsuga taxifolia has 13 chromosomes. One of the chromosomes seems to have a completely terminal fiber attachment point, 6 are heterobrachial, and 6 have approximately median fiber constrictions. The fiber attachment points are not shown clearly in the drawing (Pl. 77, fig. 21), but in other figures the shortest chromosome opens out at one end and appears to have a completely terminal attachment point.

The chromosomes of *Abies cephalonica* and *A. concolor* appear to be similar (Pl. 77, figs. 22 and 23). Five of the 12 chromosomes are clearly heterobrachial, while the others are approximately isobrachial.

In the sub-family Taxodineae the endosperm chromosomes were examined in only one genus. *Cryptomeria japonica* has 11 chromosomes in the endosperm cells. Counts were made both at metaphase and late anaphase (Pl. 77, fig. 24). All chromosomes are approximately isobrachial. Root tip counts from aceto-carmine preparations gave a diploid chromosome number of 22 in *Taxodium distichum* and in *Taiwania cryptomerioides*, but absolutely accurate counts are difficult in root tip cells, even when the cells are flattened after mounting in aceto-carmine.

In the sub-family Cupressineae three typical genera were studied. There are 11 chromosomes in *Thuja occidentalis*, in *T. orientalis*, and in *T. plicata* (Pl. 77, figs. 25, 26 and 27). One or two chromosomes are somewhat heterobrachial, and the others have approximately median fiber attachments.

Both *Juniperus virginiana* and *J. rigida* have 11 chromosomes, most of which are more or less isobrachial (Pl. 77, figs. 28 and 29). In *J. rigida* one chromosome has a small trabant. *Chamaecyparis Lawsoniana* also has 11 chromosomes in the endosperm cells (Pl. 77, fig. 30). The chromosomes resemble those of *Thuja* and *Juniperus*. In all

genera of the Cupressineae examined a few chromosomes are somewhat heterobrachial and the others approximately isobrachial.

In order to facilitate a comparison of the chromosomes in the different families and genera of Conifers, diagrams of the chromosome complements have been made for the different genera studied. The relative lengths of each chromosome are indicated by vertical lines. The relative lengths of the arms of each chromosome are shown by placing the spindle fiber attachment point on the horizontal line. The chromosomes of each genus are placed in order, according to the length of the shorter arms. These diagrams are necessarily only approximately correct, and differences between species, and in some cases differences between genera, are of doubtful significance. The graphs are reliable in indicating the more conspicuous differences in chromosome morphology. These figures are shown in diagrams on text figures 1, 2 and 3.

A survey of these figures shows that the chromosomes of *Ginkgo* are distinctly different from those of any of the Conifers. The two representatives of the Taxaceae, *Cephalotaxus*, and *Taxus* are somewhat similar in chromosome morphology, although the chromosomes of *Cephalotaxus* appear to be larger, and the short heterobrachial chromosome has a secondary constriction on the short arm. The short heterobrachial chromosome of *Taxus* has a nearly terminal fiber attachment point.

In the Pinaceae there is considerable variation in chromosome morphology between certain genera, even in the same sub-family. Little or no variation is found between diploid species of the same genus. All chromosomes of *Pinus* are approximately isobrachial. *Cedrus* differs from *Pinus* in possessing two slightly more heterobrachial chromosomes. Six of the chromosomes of *Larix* are heterobrachial. *Pseudotsuga* has 7 heterobrachial, and 5 isobrachial chromosomes, and one with a terminal fiber. *Picea* and *Tsuga* have similar chromosome morphology. *Pseudolarix* is unique among the Abietineae in having 22 chromosomes, only two of which are isobrachial. Five of the 12 chromosomes of *Abies* are distinctly isobrachial.

The chromosomes of the representatives of the Taxodineae and Cupressineae, i. e. *Cryptomeria*, *Juniperus*, *Chamaecyparis*, and *Thuja*, are similar in morphological characters, and the basic number is 11 for each genus.

MEIOTIC DIVISIONS IN CONIFERS

A study of the meiotic divisions in different genera of Conifers was undertaken to determine chromosome numbers and the frequency and

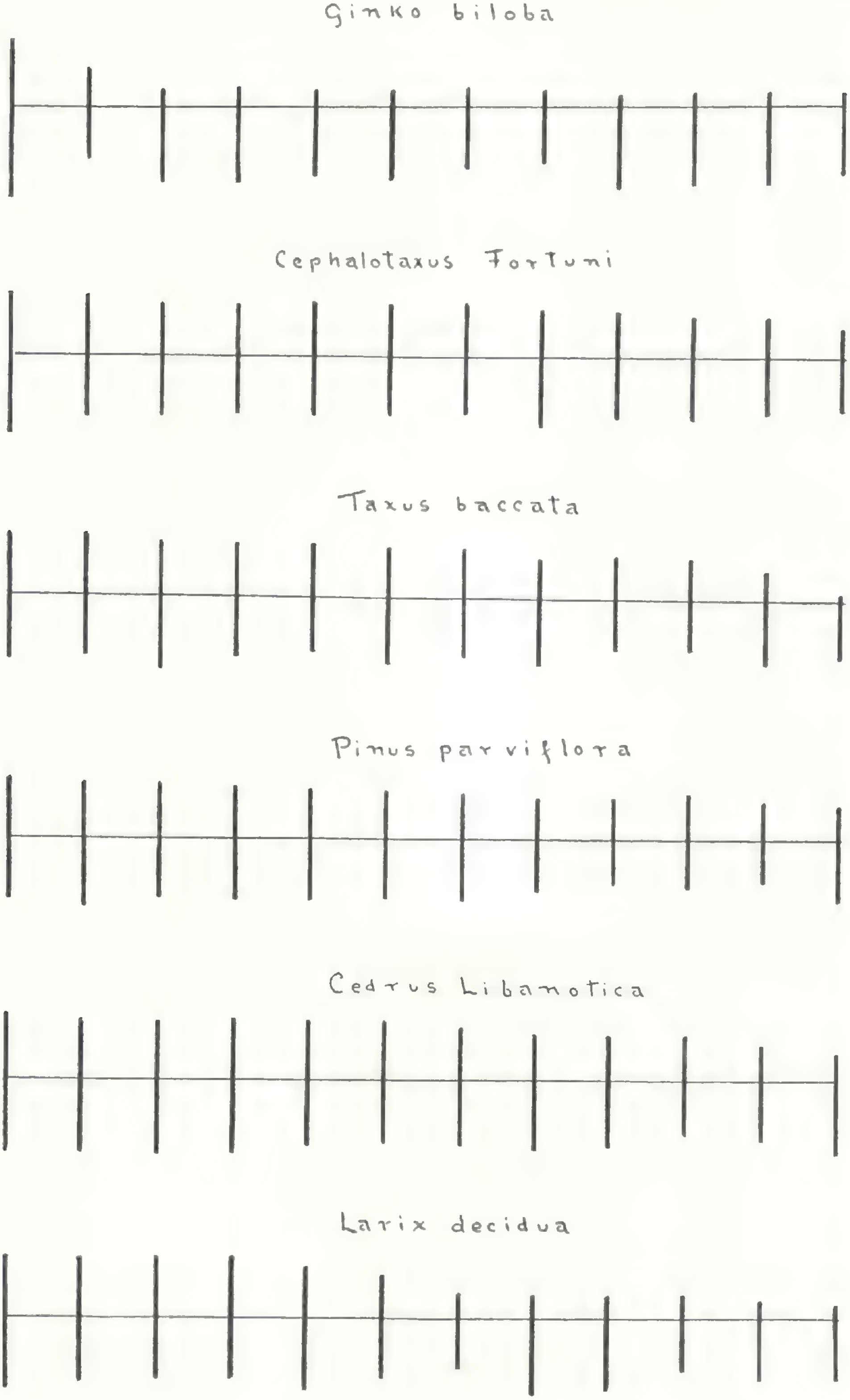

FIGURE 1. DIAGRAMS OF CHROMOSOME COMPLEMENTS. (Explanation in the text.)

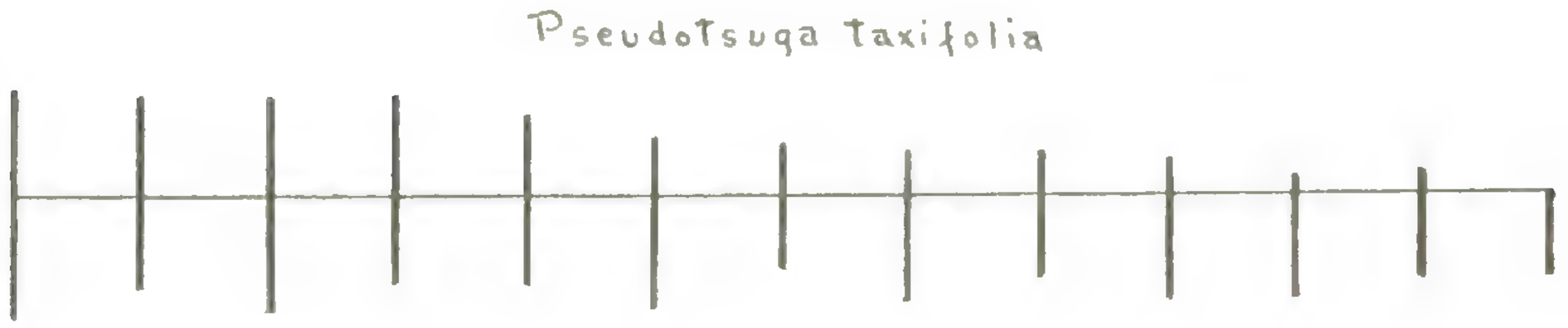

FIGURE 2. DIAGRAMS OF CHROMOSOME COMPLEMENTS. (Explanation in the text.)

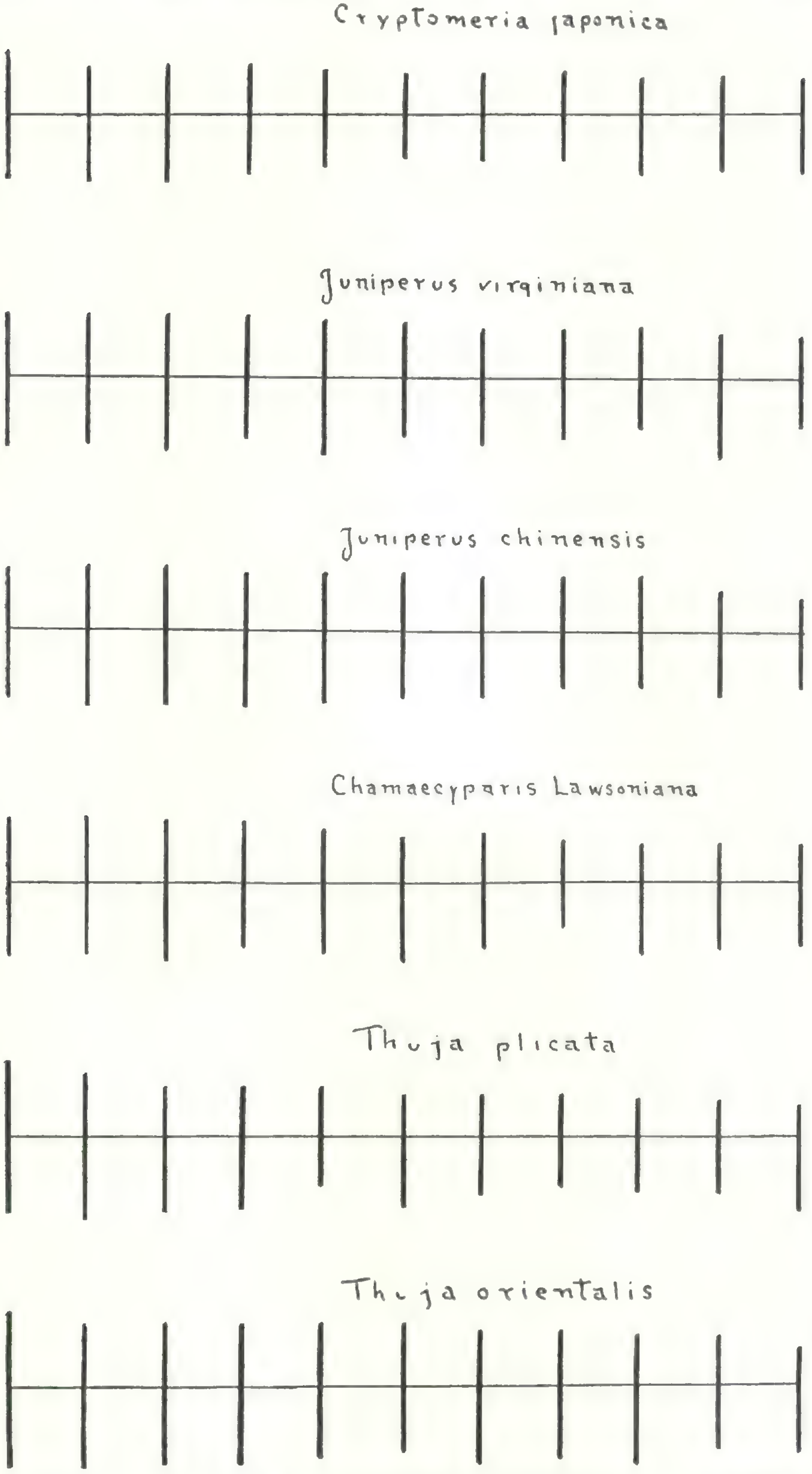

FIGURE 3. DIAGRAMS OF CHROMOSOME COMPLEMENTS. (Explanation in the text.)

types of chiasmata formed. Many of the earlier counts of chromosome numbers in Conifers were only approximations, and relatively little work has been done on the behavior of chromosomes during meiosis.

The material used for this study was obtained from Conifer species grown in the Arnold Arboretum. Chromosome counts were made from meiotic figures in microspore mother cells and divisions in the young microspores. Both aceto-carmine smears, and permanent smears stained with crystal violet, were used.

The reduction divisions in the male flowers occur at different times for different genera and species. In *Juniperus virginiana* and *J. chinensis* the reduction divisions are found in late August; in *Taxus* they occur in October; in *Larix* they are found in late February or early March, although the prophase stages begin in the fall. Reduction divisions occur in the spring in most of the other genera, and in *Juniperus rigida* and *J. communis*. In *Pinus*, few species overlap in time of meiotic divisions, and more than a month elapses between meiosis in *P. Banksiana* and *P. Strobus*. *Cedrus libanotica*, as grown in the Arnold Arboretum, is exceptional in going through meiosis early in August and shedding its pollen in early fall. The female cones develop, and seeds are produced.

The reduction divisions in many species occur almost simultaneously, and not more than two or three days elapse between the first meiotic divisions and the formation of tetrads. All trees of some species seem to undergo reduction divisions about the same time, although there is considerable variation in *Cedrus libanotica*, and in some of species of *Juniperus*.

Both aceto-carmine and permanent smears provided good preparations for a study of chromosome numbers and chiasma frequency. The chiasma frequency does not seem to be very high at mid-diplotene, and most of the chiasmata are not completely terminalized at early metaphase. There are usually somewhat more than two chiasmata per bivalent at metaphase, and these are usually interstitial or sub-terminal. The various stages in meiosis have been photographed to show the typical behavior of the chromosomes in Conifer species. The figures shown in plate 78 are from *Picea Abies*. The double nature of the pachytene "spireme" is not clearly shown in the photograph (Fig. 31), but when flattened, the threads do show the paired chromomeres. The early diplotene stages are not sufficiently distinct for a study of chiasma formation, but at mid-diplotene the chromosomes are clearly defined

in many cases (Fig. 32). The average chiasma frequency at this time is about three per bivalent. At metaphase most of the bivalents are in the form of rings with segments usually projecting beyond the interstitial or subterminal chiasmata. Occasional rod bivalents are found at late metaphase (Fig. 33). As the bivalents divide, their tetrad nature is evident, and the relations of the four chromatids often can be determined in permanent preparations. Frequently two chromatids retain their terminal association after the others have separated (Fig. 34). The chromosomes are easily counted either at metaphase or late anaphase (Fig. 35). A telophase stage of the second division is shown in figure 36. The meiotic divisions appear to be normal, and there is no evidence of chromosome irregularity.

The chiasma frequencies in the chromosomes of different genera of Conifers, and especially in the Abietineae, seem to be very similar. Photographs of meiotic figures of six different genera of Abietineae are shown in plate 79. The meiotic chromosomes of *Larix* have about 2.4 chiasmata per bivalent, as was determined earlier in two species and the F_1 hybrid (Sax, 1932). Most of the chiasmata at diakinesis are interstitial (Pl. 79, fig. 37), and the free distal arms are widely separated. Essentially the same configurations are found in *Pinus* (Pl. 79, fig. 38). There is little terminalization of chiasmata between diakinesis and early metaphase. The first meiotic metaphase in *Tsuga* is shown in Pl. 79, fig. 39. The analysis of chromosome morphology in Pseudolarix endosperm showed a total of 22 chromosomes, of which 20 have terminal or subterminal fiber constrictions. The bivalents at meiosis are of the ring-rod type, usually with two chiasmata at the same side of the attachment points (Pl. 79, fig. 40). The meiotic chromosomes of *Cedrus* (Pl. 79, fig. 41) and of *Abies* (Pl. 79, fig. 42) have approximately the same chiasma frequencies as those of *Picea, Larix,* and *Pinus*. In general, there is a striking similarity in the meiotic figures of the different diploid Conifers.

A detailed study of chiasma frequency at meiosis has been made for 22 species of Conifers. The only genus represented in the Taxaceae is *Taxus;* but in the Pinaceae two of the sub-families are represented, and all genera of the Abietineae, with the exception of *Keteleeria,* have been examined. The chiasma frequencies are shown in the following table (Table I). Authorities for species names are those given by Rehder (1927).

TABLE I.

CHROMOSOME NUMBER AND CHIASMA FREQUENCY

Species	Chromsome Number	Chiasma frequency 0	1	2	3	4	5	Ave. xta frequency per bivalent
Abies cephalonica	12		8.2	55.1	28.9	7.6		2.4
Abies Nordmanniana	12		6.5	55.9	29.6	8.8		2.4
Cedrus libanotica	12		.8	58.6	29.7			2.5
Juniperus communis	11		7.5	63.3	24.1	5.0		2.2
Larix decidua	12		4.0	63.0	25.0	8.0		2.4
Larix eurolepis	12	0.7	8.0	48.0	32.0	10.7	0.1	2.4
Larix Kaempferi	12		10.0	40.0	25.0	18.0	0.7	2.5
Picea Abies	12		1.7	50.8	30.8	10.0	6.7	2.7
Picea mariana	12		2.7	42.3	43.0	11.1	0.7	2.6
Pinus Banksiana	12		8.3	58.3	26.1	7.2		2.3
Pinus Jeffreyi	12	0.6	7.7	60.1	25.6	5.9		2.4
Pinus nigra	12		6.5	55.5	29.6	8.3		2.4
Pinus Strobus	12		4.2	53.7	29.6	12.5		2.5
Pinus Thunbergiana	12		4.5	61.4	26.5	7.6		2.4
Pseudolarix amabilis	22	0.7	8.7	67.5	19.9	3.1		2.1
Taxus cuspidata	12	0.4	15.1	71.1	12.3	0.9		2.0
Taxus Hunnewelliana	12		15.3	79.5	5.1			1.9
Taxus media	12	5.0	8.8	83.0	7.7			2.0
Thuja Standishii	11		9.2	75.0	15.8			2.1
Tsuga canadense	12		9.1	77.2	12.8	0.7		2.1
Tsuga caroliniana	12	0.6	7.1	57.0	25.6	9.6		2.4
Tsuga diversifolia	12		6.1	70.0	21.6	1.6		2.2

In the above table the proportion of chromosomes with a given chiasma frequency is given in percentage. This was done to facilitate comparisons of chiasma frequencies in different species. With one exception all chiasma frequencies were based on at least 10 pollen mother cells which, in most species, involved 120 or more bivalent chromosomes.

The chiasma frequencies are similar for all genera, ranging from 1.9 to 2.7 chiasmata per bivalent chromosome. The species within each genus have essentially the same chiasma frequencies, and the differences found are probably not significant. The chiasma frequency found in *Taxus* agrees with Dark's (1932) observations.

The meiotic divisions were very regular, and unpaired chromosomes were found only in rare cases. Even the species hybrids show regular divisions and a high percentage of fertile pollen. The pollen sterility was determined for all species used in the analyses of chiasma frequencies and for most of those used for studies on somatic chromosomes. In most cases the pollen sterility ranged from less than 1 percent to 5 percent. The species with more than 5 percent pollen sterility included *Abies cephalonica* (10%), *A. Nordmanniana* (7%), *Juniperus chinensis* (10%), *J. communis* (18%), *Taxus cuspidata nana* (8%), and *T. media* (9%).

There is evidence of well defined polarity in the development of the microspores of Conifers. In the winged pollen grains, the first division in the microspore is at right angles to the axis of the wings and near the heavy wall of the cell. The nucleus nearer the wall disintegrates and the other divides. Again the nucleus near the wall disintegrates, and the remaining nucleus divides to form the tube and generative nuclei. The nucleus nearer the cell wall becomes the generative nucleus which ultimately produces the male gametes. In rare cases the divisions are oriented parallel to the axis of the wings. In such cases four nuclei of equal size are produced, and there is no evidence of any differentiation. Apparently nuclear differentiation in these microspores is dependent on polarity.

Chromosome numbers were also determined in a number of species where adequate material was not available for a study of chiasma frequencies. The number of bivalents found at meiosis was 12 in *Abies Veitchii, Larix occidentalis, Picea glauca, Pinus echinata, P. ponderosa, P. pungens, P. rigida, P. sylvestris, P. tabulaeformis, P. virginiana* and *Taxus canadensis*. *Juniperus chinensis Pfitzeriana* is undoubtedly a tetraploid with about 22 pairs of chromosomes. All other species of *Juniperus* studied have 11 pairs of chromosomes. *Cryptomeria japonica* also has 11 pairs of chromosomes at meiosis.

DIFFERENTIATION OF SPECIES AND GENERA OF CONIFERS

The basic chromosome number seems to be 12 for most Conifers and other typical Gymnosperms. The chromosome counts listed by Tischler (1927, 1931) include 8 genera of Cycadaceae, all of which have 12 chromosomes, with one doubtful exception. Several investigators have found 12 pairs of chromosomes in *Ginkgo*. Less than 12 chromosomes have been found in *Taxus* and *Torreya* by earlier workers, but Ishikawa's count of 12 in *Taxus* has been confirmed by the

work of Dark and by our studies. Burlingame, Ishikawa, and Schurhoff have found 12 chromosomes in several species of *Podocarpus*. Twelve is the basic number reported in *Larix, Pinus, Picea, Tsuga,* and *Abies*. The earlier counts in the Araucarineae, Cupressineae, and Taxodineae were obtained in connection with morphological studies, and for the most part they are of doubtful value. The Gnetales are exceptional types of Gymnosperms and seem to be exceptional in chromosome number. The most reliable counts in this family seem to be those of Geitler, who finds 7 pairs of chromosomes in *Ephedra*. Twelve appears to be the original basic number for all other Gymnosperms.

Deviations from the typical basic chromosome number are found in the Conifers, but they can be attributed to the addition or loss of a chromosome, or to polyploidy. Thirteen chromosomes are found in *Pseudotsuga taxifolia* endosperm cells. Meiotic divisions were not observed in this genus, so that it is not possible to say whether the extra chromosome is a simple duplication, or a duplication followed by interchange of segments and loss of complete homology with any one of the twelve chromosomes of the basic complement.

Cryptomeria, Thuja, Juniperus, and *Chamaecyparis* each have 11 chromosomes as a basic number. If 12 is the basic chromosome number in the Conifers, it is improbable that the lower number is the result of a loss of an entire chromosome. The loss of a single chromosome from the basic complement produces inviable gametes and zygotes in practically all plants. It seems more probable that interchange of segments left one chromosome too short to pair regularly. If such a chromosome carried only unessential genes, it could be eliminated with no lethal effect on either gametes or zygotes.

Pseudolarix has two isobrachial chromosomes and twenty with terminal or subterminal fiber attachment points. The chromosome morphology of this species might suggest that the original chromosome complement consisted of twelve more or less isobrachial chromosomes, of which ten had segmented at or near the fiber attachment. This suggestion is in accord with the fact that there are few multivalent associations of chromosomes at meiosis. An increase in chromosome number by fragmentation would also necessitate a corresponding increase in fiber attachment points, and there is no evidence that such attachment points can be divided or formed *de novo*. To be sure Darlington (1929) has postulated that chromosome fragments of a certain size may form new fiber attachments, but as Navashin (1932) has pointed out, the evidence against this hypothesis is very conclusive.

Chromosome fragments of any size do not survive unless they retain the fiber attachment or are attached to another chromosome possessing it. It is improbable, therefore, that the chromosome number of *Pseudolarix* can be attributed to fragmentation. If *Pseudolarix* is a polyploid with twelve chromosomes as the original basic number, the aneuploid condition caused by the loss of two chromosomes would not necessarily be deleterious, especially if preceded by segmental interchange. Interchange between many of the remaining non-homologous chromosomes might reduce homology to such an extent that few multivalent associations would be found at meiosis, even though the chiasma frequency exceeds two per bivalent. Homology of several chromosomes in the haploid complement is shown in dividing figures in endosperm tissue. In one metaphase figure two chromosomes were in contact at all loci, and several other chromosomes were found closely associated in pairs.

Polyploidy is rare in the Conifers and has been reported previously only in *Sequoia sempervirens*. Dark (1932) found about 50 chromosomes in the root tips of this species and considers it to be a tetraploid. Our aceto-carmine preparations of root tips also show that this species is a polyploid. Alhough the exact number of chromosomes could not be determined, there were more than 40. The somatic number in *Sequoia gigantea* is from 21 to 24 chromosomes, according to Goodspeed and Crane (1920). *Juniperus chinensis Pfitzeriana* is undoubtedly a tetraploid with 22 pairs of chromosomes. The other Juniper species examined have 11 pairs of chromosomes. The three polyploid species of Conifers described are presumably auto-polyploids, although changes in chromosome morphology may have reduced homology subsequent to chromosome duplication.

It is evident that changes in chromosome number have played a minor part in the differentiation of genera of Conifers. The Cupressineae differ from most other Conifers in having a basic chromosome number of 11. The closely related sub-family Taxodineae may also have 11 chromosomes as the basic number. According to Dark (1932), *Cryptomeria* has 24 somatic chromosomes, but counts from root tips are unsatisfactory and may not be reliable. We have found 11 chromosomes in the endosperm cells of *Cryptomeria* and in meiotic figures. Coker (1903) pictures 11 chromosomes in the microspore division in *Taxodium*. Goodspeed and Crane (1920) found 21 to 24 chromosomes in root tips of *Sequoia gigantea*, which might be taken to mean that the somatic number was less than 24. We have also found about 22 somatic chromosomes in *Taiwania* and *Taxodium*.

Differentiation of genera of Conifers seems to be associated, to a considerable extent, with differences in chromosome morphology. The two genera of Taxaceae studied are somewhat similar in *chromosome* morphology, although two of the chromosomes of *Taxus* are more nearly subterminal than those of *Cephalotaxus*. The Abietineae show considerable variation. All chromosomes of *Pinus* are approximately isobrachial; one chromosome of *Cedrus* is distinctly heterobrachial; six chromosomes of *Larix* are heterobrachial; and all but two of the twenty-two chromosomes of *Pseudolarix* have terminal or subterminal fiber attachments. *Picea, Abies* and *Tsuga* show some variation in length of chromosomes and positions of fiber attachments. In the Cupressineae, however, there is little difference in chromosome morphology of different genera. The chromosomes of *Juniperus, Thuja,* and *Chamaecyparis* are similar in morphology and are all more or less isobrachial. The chromosomes of *Cryptomeria,* representing the Taxodineae, resemble those of the Cupressineae. These two sub-families seem to be similar in chromosome number and chromosome morphology.

Where different genera show well marked differences in chromosome morphology, one might suppose that such variation is responsible for the initial isolation which would permit independent variation and the ultimate differentiation of genera. If changes in chromosome morphology are caused primarily by segmental interchange, as seems probable, then the new interchange types may be isolated from the original type because of the sterility of individuals heterozygous for segmental interchange. A high degree of sterility in individuals heterozygous for segmental interchange chromosomes would be expected in the Conifers because of the prevalence of interstitial chiasmata at meiosis. The rigidity of interchange rings caused by interstitial chiasmata would result in a high percentage of non-disjunction and consequently a high gametic sterility (Sax and Anderson, 1933). Only the forms homozygous for segmental interchange could survive, and variations produced in these forms would not be swamped by intercrossing with the original parental types with a different chromosome morphology. A considerable amount of interchange and natural selection would have to occur to produce the variations found in certain genera.

Differentiation of genera, and even sub-families, in the Cupressineae and Taxodineae has occurred with little apparent change in chromosome morphology. Exchange of approximately equal segments could occur with no apparent change in chromosome morphology, as is the case in *Tradescantia* (Sax and Anderson, 1933). On the other hand,

it is quite possible that the differentiation of these genera may be initiated and maintained by genetic factors, as seems to be the case in certain races and species of *Drosophila* (Schultz and Dobzhansky, 1933; and Dobzhansky, 1933).

The factors involving the differentiation of genera in the Conifers seem to be of a different nature from those responsible for species differentiation. In the Abietineae, especially, the genera differ considerably in chromosome morphology, but the species within a genus show little or no variation in chromosome morphology. If species differentiation in the Conifers is caused primarily by gene mutations, many species within a genus may not differ sufficiently to prevent species hybridization and fertility of the F_1 hybrids. The available evidence seems to support this assumption. *Larix eurolepis* is an F_1 hybrid between *L. Kaempferi,* a native of Japan, and the European Larch, *L. decidua.* Although the parental species have been isolated for a long time, the F_1 hybrid shows almost complete chromosome pairing, the chiasma frequency at meiosis is as high as that found in the parents, and more than 90 percent of the pollen is good (Sax, 1932). According to Rehder (1927), *Taxus media* is a hybrid of the Japanese Yew, *T. cuspidata,* and the English Yew, *T. baccata. Taxus Hunnewelliana* is supposed to be a hybrid between *T. cuspidata* and *T. canadensis.* Both of these hybrids show regular chromosome pairing, normal chiasma frequency at meiosis, and a high degree of fertility. Species hybrids have been found in *Abies, Tsuga, Picea, Larix,* and *Pinus* (Rehder, 1927). According to Austin (1927), there are seven known natural species hybrids in *Pinus* and two of artificial origin. Austin has made additional crosses which seem to be successful.

If different species cross readily, there must be some factors which prevent extensive crossing and thus maintain the species as units. Two factors certainly play a part in species isolation,—geographic distribution and time of flowering. Although the ranges of certain species overlap, many species are so separated in distribution that crossing could never occur in nature. The shedding of pollen in most species is completed in a few days, and there is often no overlapping of flowering periods within species of a given genus. For example, *Tsuga canadensis* sheds its pollen before *T. caroliniana* or *T. diversifolia.* The last two species flower at the same time in the Arboretum, but in nature they are geographically isolated. Artificial crosses have been made between these two species which have resulted in an abundant set of seed. The species of *Pinus* also show a great range in time of flower-

ing, and more than a month elapses between the pollination of *P. Banksiana* and pollination of *P. Strobus*. Many species of *Pinus* are undoubtedly interfertile, but certain species in different sections may be so genetically differentiated that the artificial production of hybrids would be difficult or impossible.

The chromosome numbers in the Gymnosperms are of interest in regard to the absence of double fertilization in this group of plants. According to Müntzing (1933), hybrid incompatibility and the occurrence of polyploidy in the Angiosperms are more or less dependent on double fertilization. Polyploidy is preserved because of incompatibility between diploids and polyploids, caused by the disturbed relations between embryo, endosperm, and somatic tissues. As evidence in support of this view, Müntzing points out the rare cases of polyploidy in genera which do not have double fertilization.

Although polyploidy is rare in the Conifers, at least two polyploid species are known, *Juniperus chinensis Pfitzeriana* and *Sequoia sempervirens*, and it is probable that *Pseudolarix*, also, is of polyploid origin. One might suppose that the prevalence of interstitial chiasmata in the Conifers would result in quadrivalent chromosome configurations at meiosis of such a nature that irregular chromosome distribution would occur and produce considerable pollen sterility, but the pollen sterility in the tetraploid Juniper is only 6 percent, which is less than that found in some of the diploid species. There is less than 5 percent of pollen sterility in *Pseudolarix*. It is evident that the rare occurrence of polyploidy in the Conifers can not be attributed to the sterility of such types.

According to Müntzing's theory polyploid Conifers are rare because diploid and polyploid forms can cross readily, and consequently the polyploid form is not isolated and can not develop independently unless geographically isolated. This hypothesis might also explain the differentiation of relatively few species and genera in the Gymnosperms.

SUMMARY

The authors have determined the chromosome numbers for 53 species representing 16 genera of Conifers. The basic chromosome number is 12 for most Gymnosperms, with the exception of the Gnetales. Twelve pairs of chromosomes have been found in all genera studied in the Taxaceae. Deviations from the typical basic number are found in the Pinaceae. In the Abietineae 12 pairs of chromosomes are found in *Picea, Tsuga, Abies, Larix,* and *Pinus,* as reported by earlier investi-

gators. *Cedrus* also has 12 chromosomes, but in *Pseudotsuga* there are 13, and in *Pseudolarix* there are 22 pairs of chromosomes. The basic number is 11 in the Cupressineae as represented by *Thuja, Juniperus,* and *Chamaecyparis*. One variety of *Juniperus chinensis* is a tetraploid. The basic number seems to be 11 in the Taxodineae examined also. *Cryptomeria* undoubtedly has 11 pairs of chromosomes, and the same number was found in *Taxodium* and *Taiwania*. *Sequoia sempervirens* is a polyploid, but the exact number of chromosomes could not be determined. Deviations from the primary basic number are attributed to the loss of a small chromosome, following translocation of segments, in the Cupressineae and Taxodineae; duplication of a chromosome in *Pseudotsuga;* and polyploidy in three species of Conifers.

Genera of Conifers may differ considerably in chromosome morphology, but species within each genus are very similar. Differentiation of genera is often associated with changes in chromosome morphology, presumably caused by segmental interchange. Species differences seem to be based primarily on genic changes, which, in many cases, do not prevent compatible species hybrids. Many species maintain themselves as distinct units only by geographic or physiological isolation.

The chiasma frequency and behavior of the chromosomes at meiosis has been determined for 22 species representing 10 genera of Conifers. The average number of chiasmata per bivalent ranges from about 2.0 to 2.7. The meiotic figures are somewhat similar in all diploid species examined, and especially so in the Abietineae. The similarity in chromosome numbers and meiotic configurations in the Conifers is remarkable as contrasted with the great variation in numbers and chiasma frequencies found in the Angiosperms.

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EXPLANATION OF THE PLATES

Plate 75.

Photographs of different stages in thè mitotic cycle in the endosperm of *Cephalotaxus Fortuni*.

Fig. 1. Prophase showing "spireme" and single nucleolus.
Fig. 2. Metaphase. The fiber attachment points can be seen in several chromosomes.
Fig. 3. Anaphase. Separation of daughter chromosomes almost complete.
Fig. 4. Late anaphase. The number and types of chromosomes are easily determined.
Fig 5. Late telophase. The chromosomes form a compact mass at each pole.
Fig. 6. Reconstruction of daughter nuclei. The chromosomes are polarized around a single nucleolus.

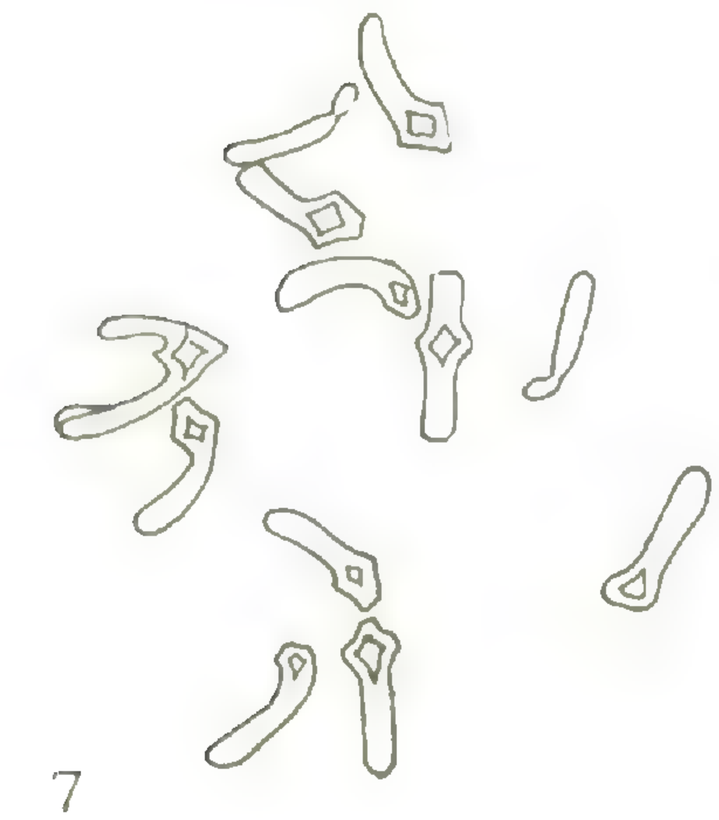
Plate 76.

Somatic chromosomes from endosperm tissue of Conifers. Drawn from aceto-carmine preparations, × 1200.

Fig. 7. *Ginkgo biloba.* Metaphase. 12 chromosomes.
Fig. 8. *Taxus canadensis.* Metaphase. 12 chromosomes.
Fig. 9. *Taxus baccata.* Metaphase. 12 chromosomes.
Fig. 10. *Taxus cuspidata.* Metaphase. 12 chromosomes.
Fig. 11. *Pinus parviflora.* Metaphase. 12 chromosomes.
Fig. 12. *Pinus Thunbergiana.* Metaphase. 12 chromosomes.
Fig. 13. *Pinus ponderosa.* Metaphase. 12 chromosomes.
Fig. 14. *Cedrus libanotica.* Metaphase. 12 chromosomes.
Fig. 15. *Larix Kaempferi.* Late anaphase. 12 chromosomes.
Fig. 16. *Larix decidua.* Metaphase. 12 chromosomes.
Fig. 17. *Pseudolarix amabilis.* Metaphase. 22 chromosomes.
Fig. 18. *Picea pungens.* Metaphase. 12 chromosomes.

Conifer Chromosomes

THE HELIOTYPE CORP. BOSTON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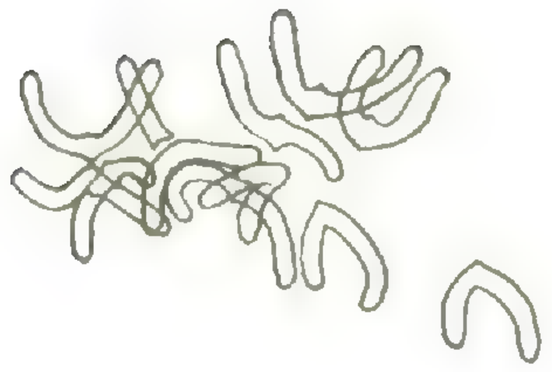
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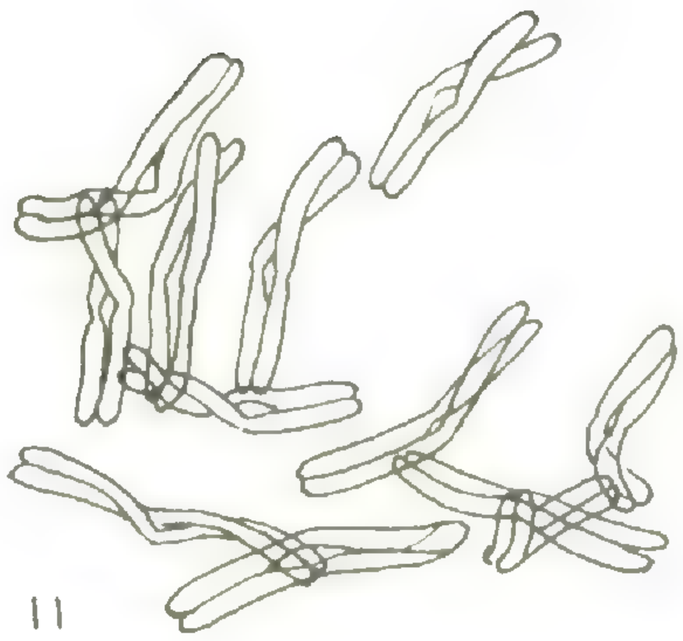
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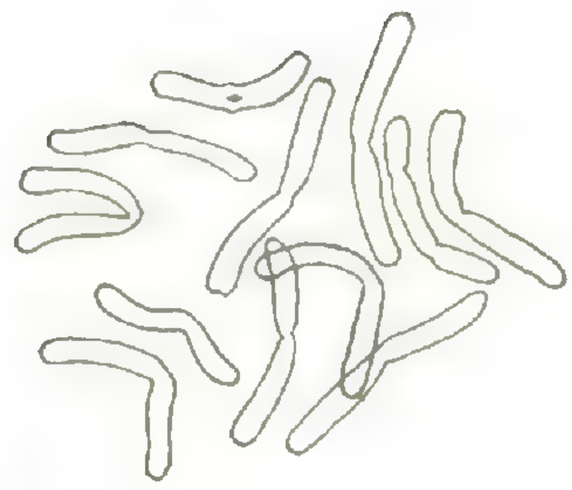
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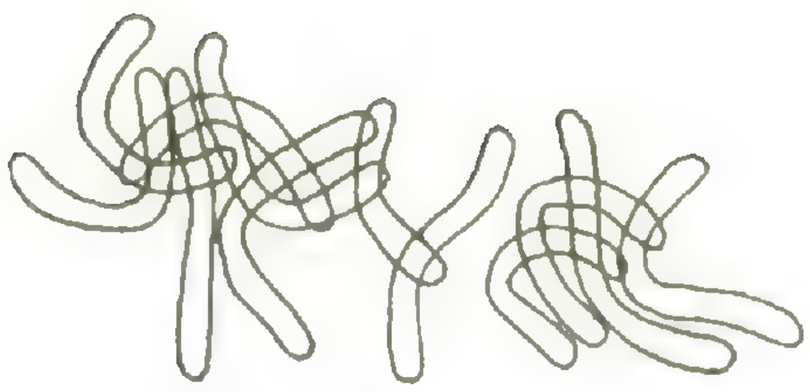
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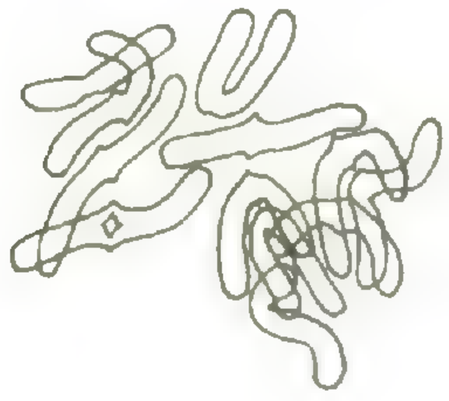
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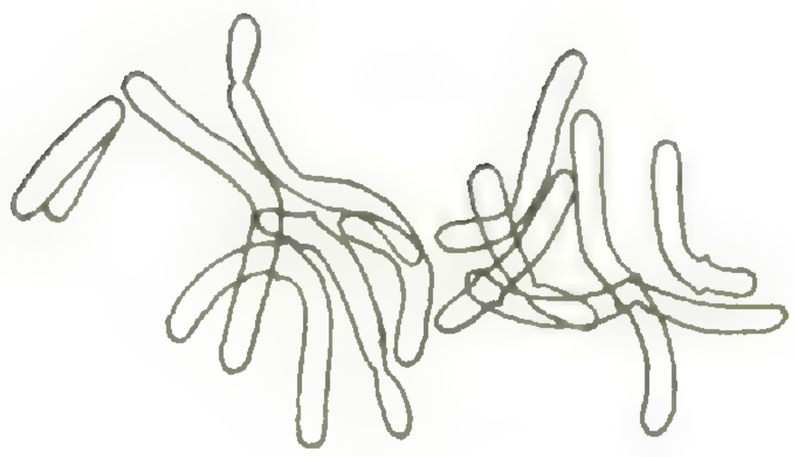
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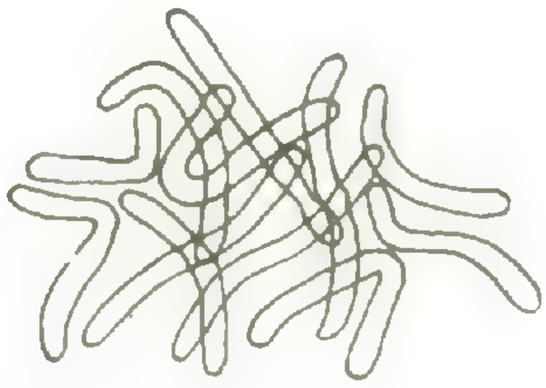
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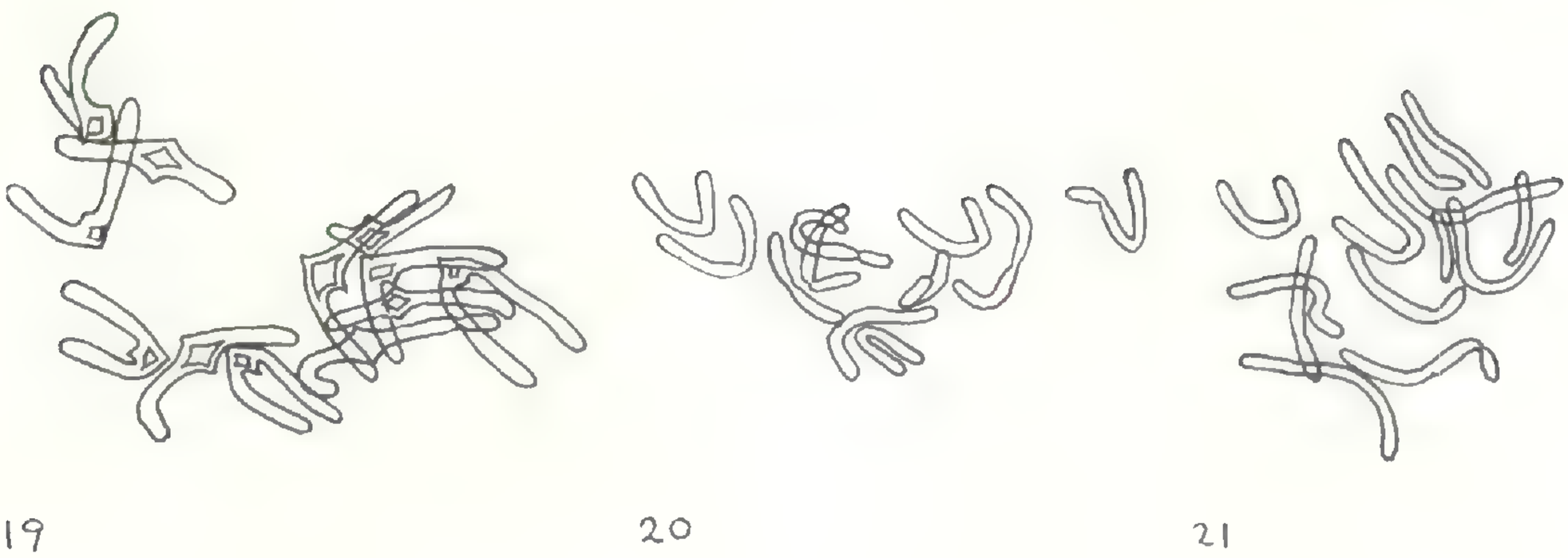
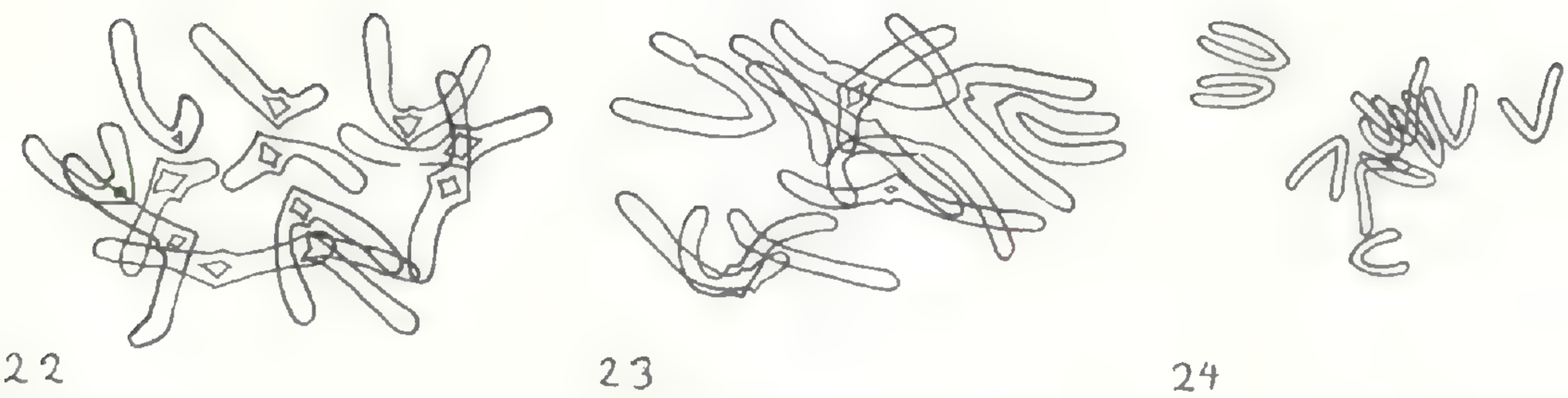
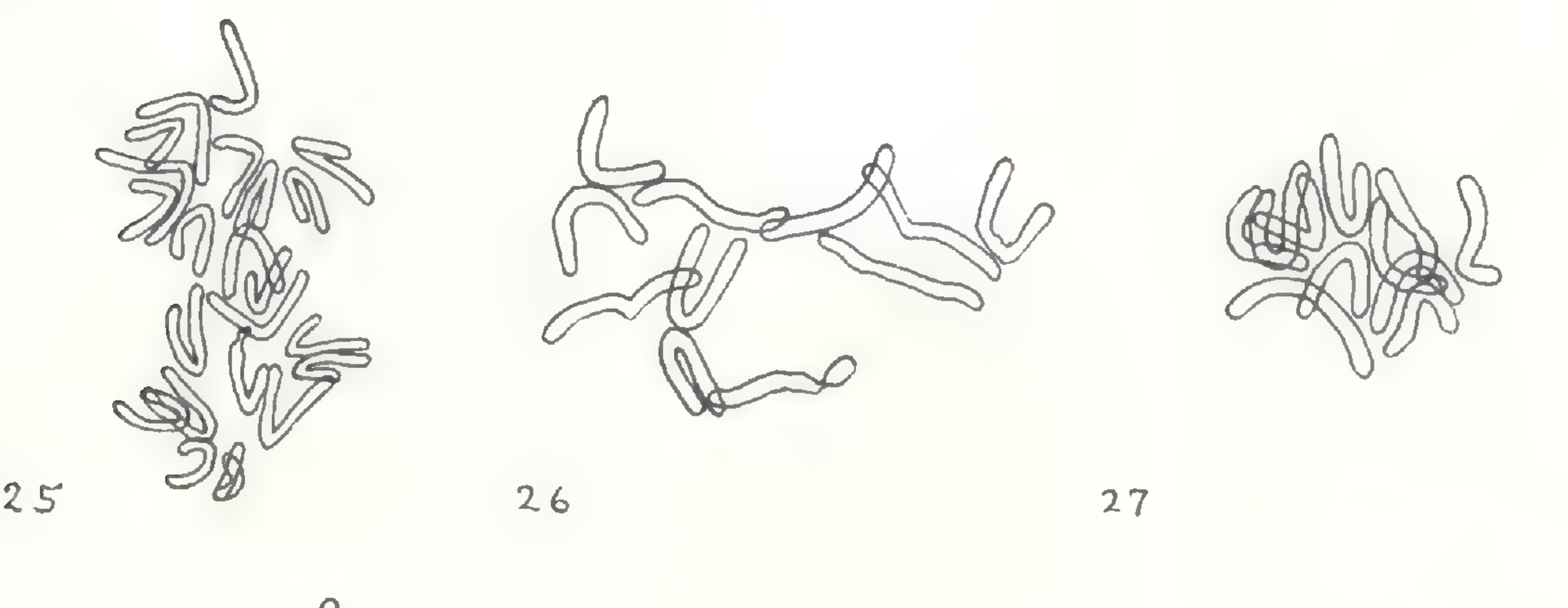
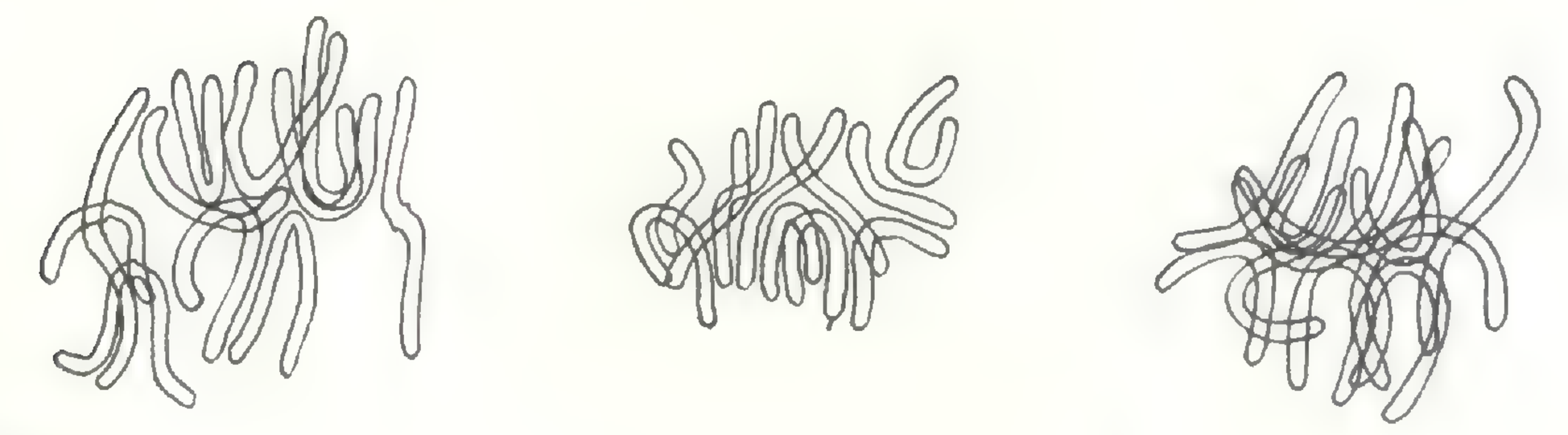
Conifer Chromosomes

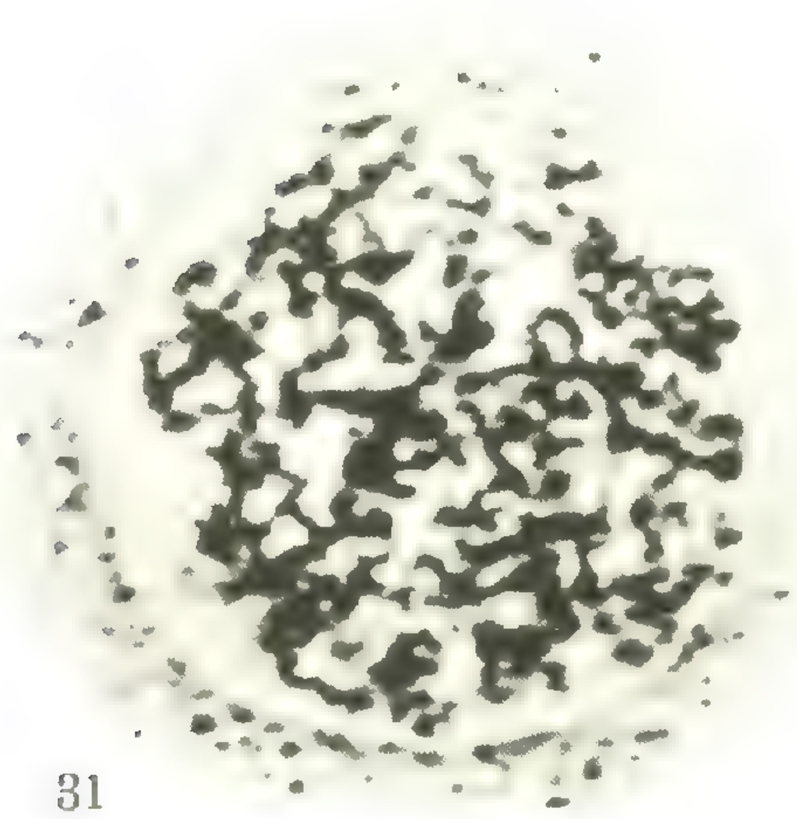








CONIFER CHROMOSOMES



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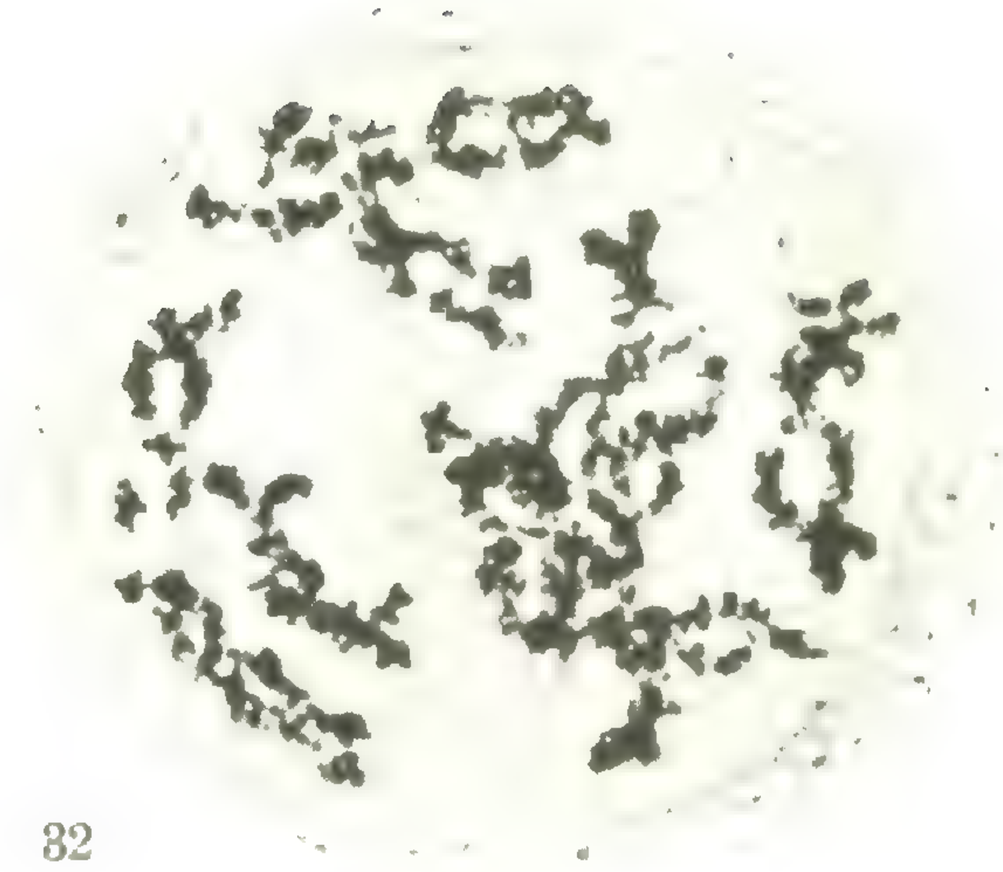

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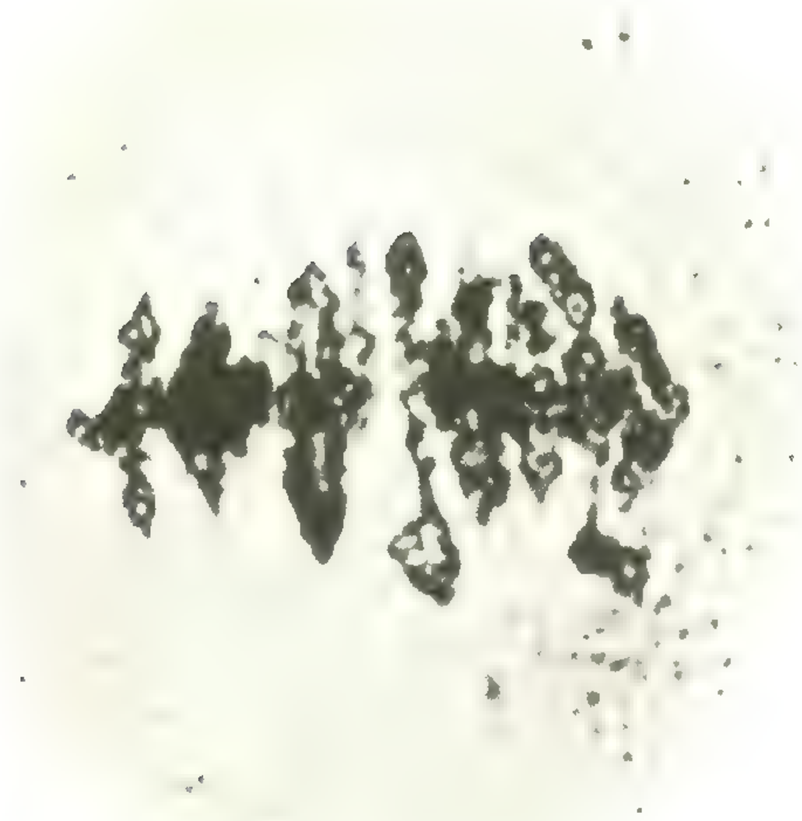

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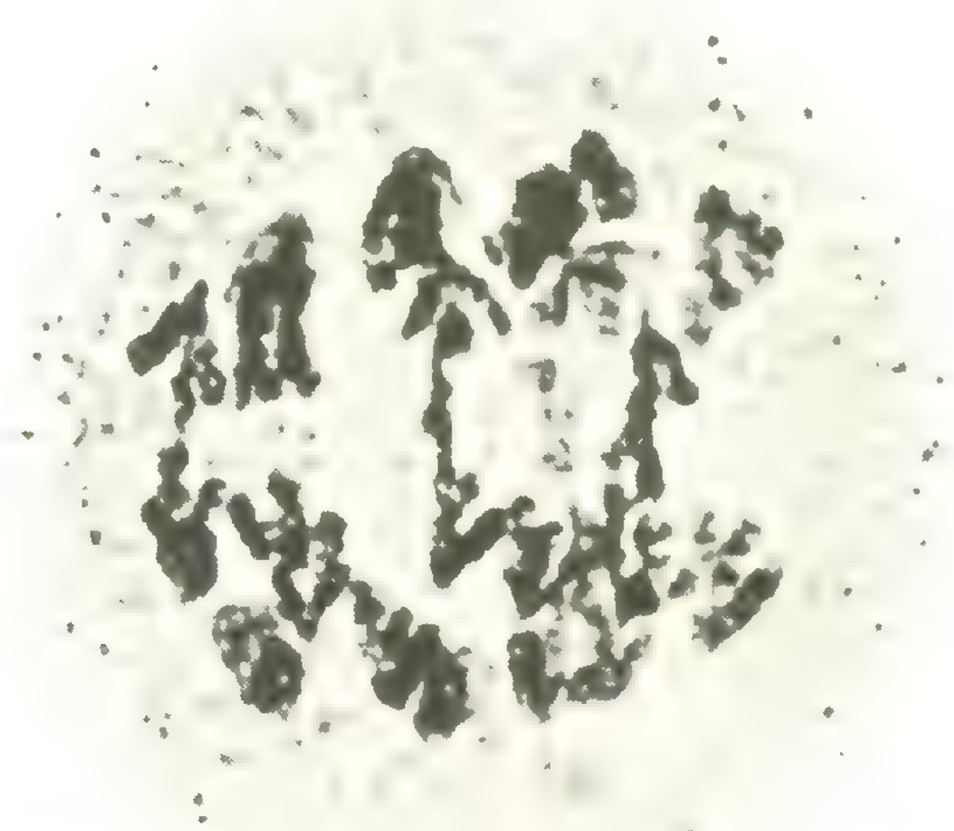

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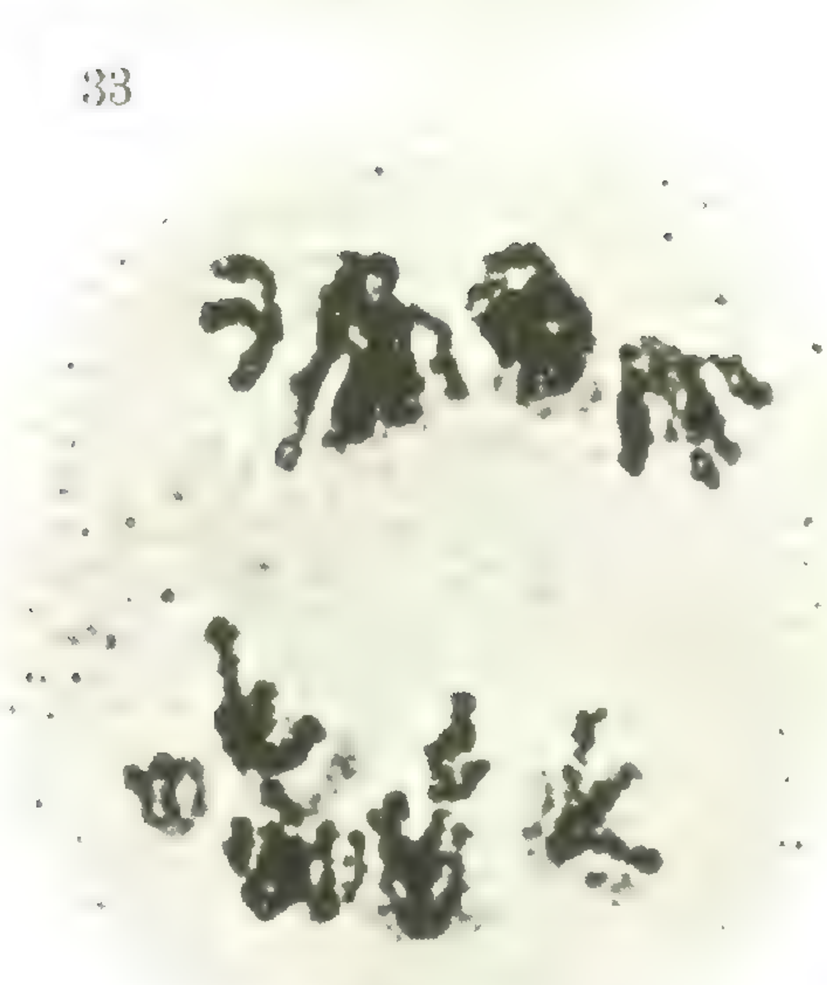

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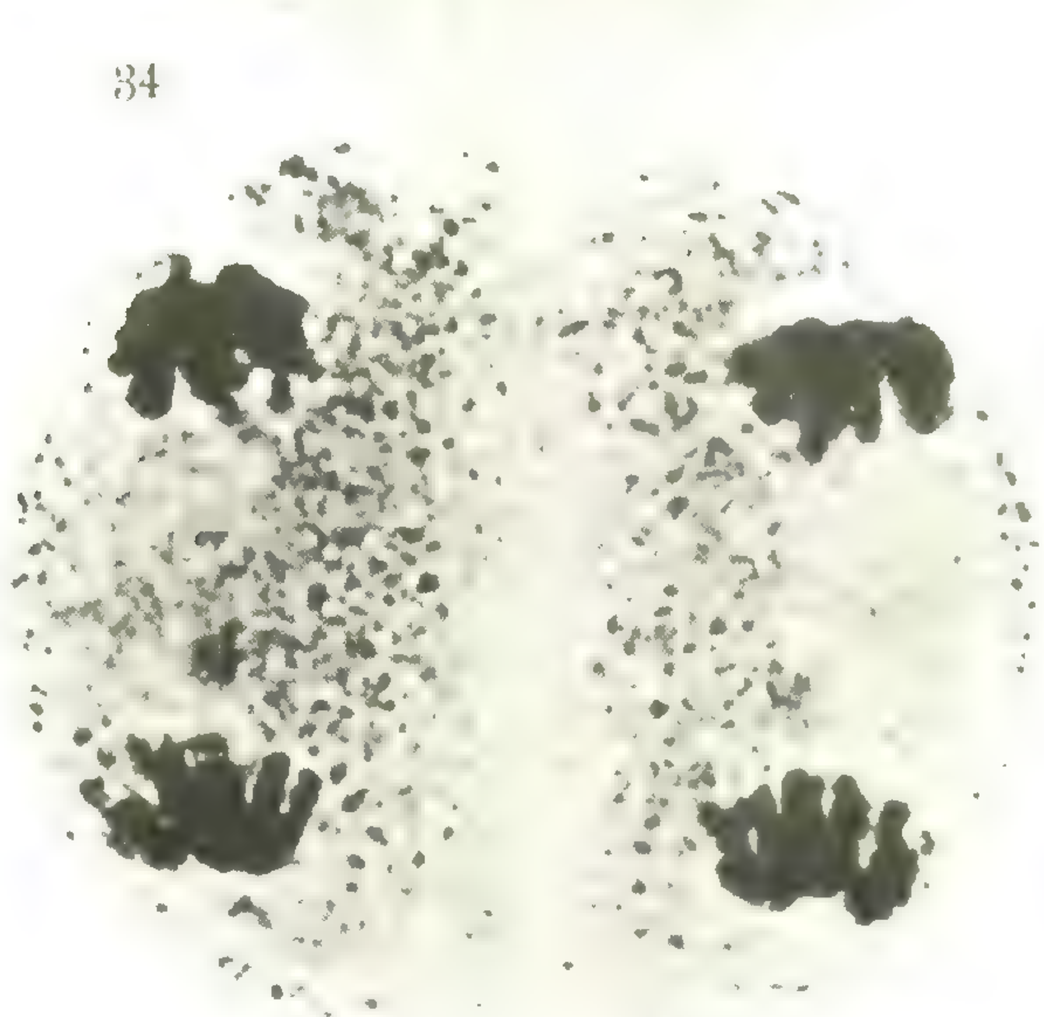

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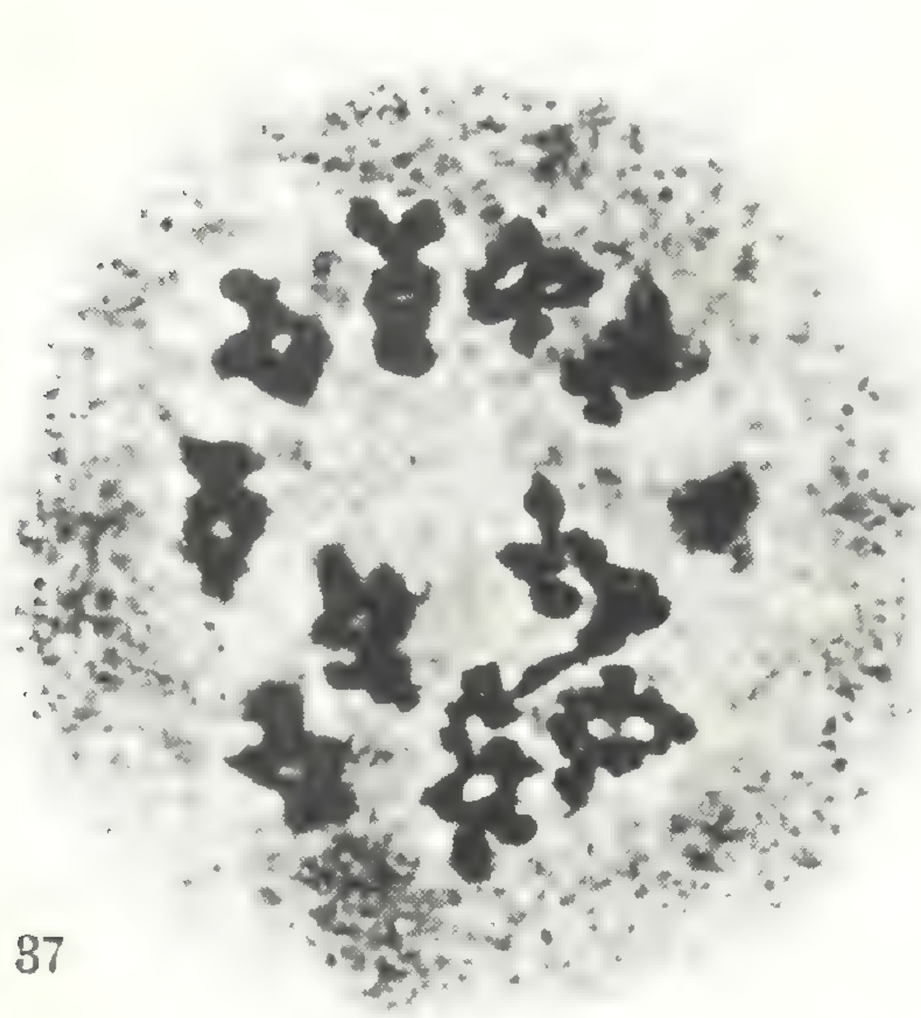
Conifer Chromosomes

THE HELIOTYPE CORP. BOSTON



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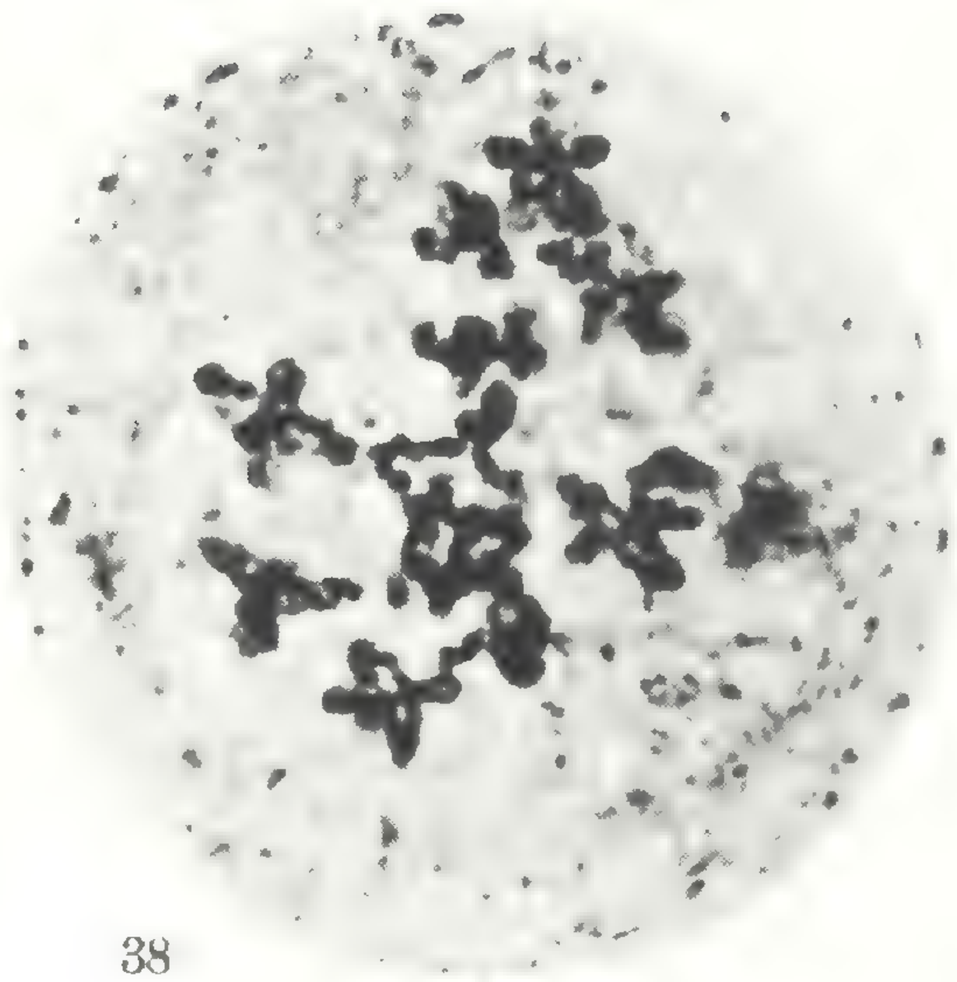

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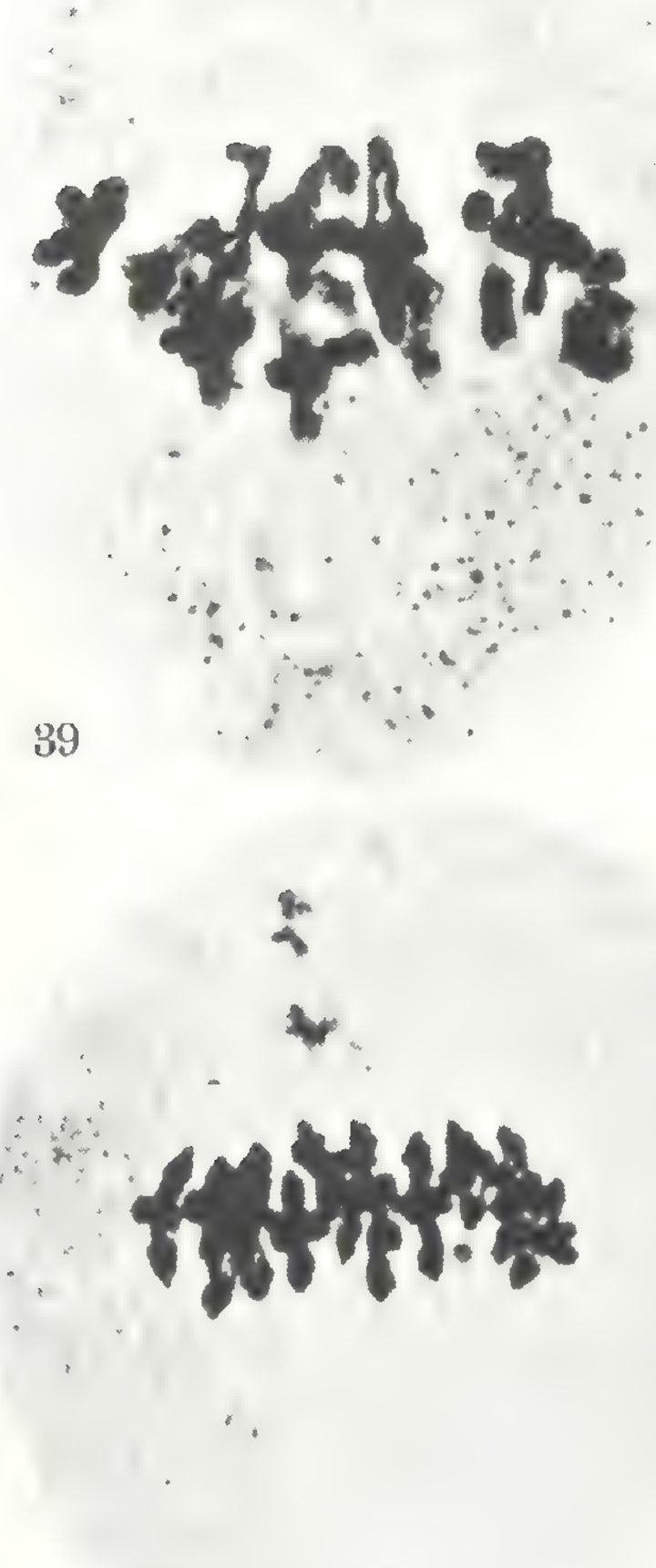

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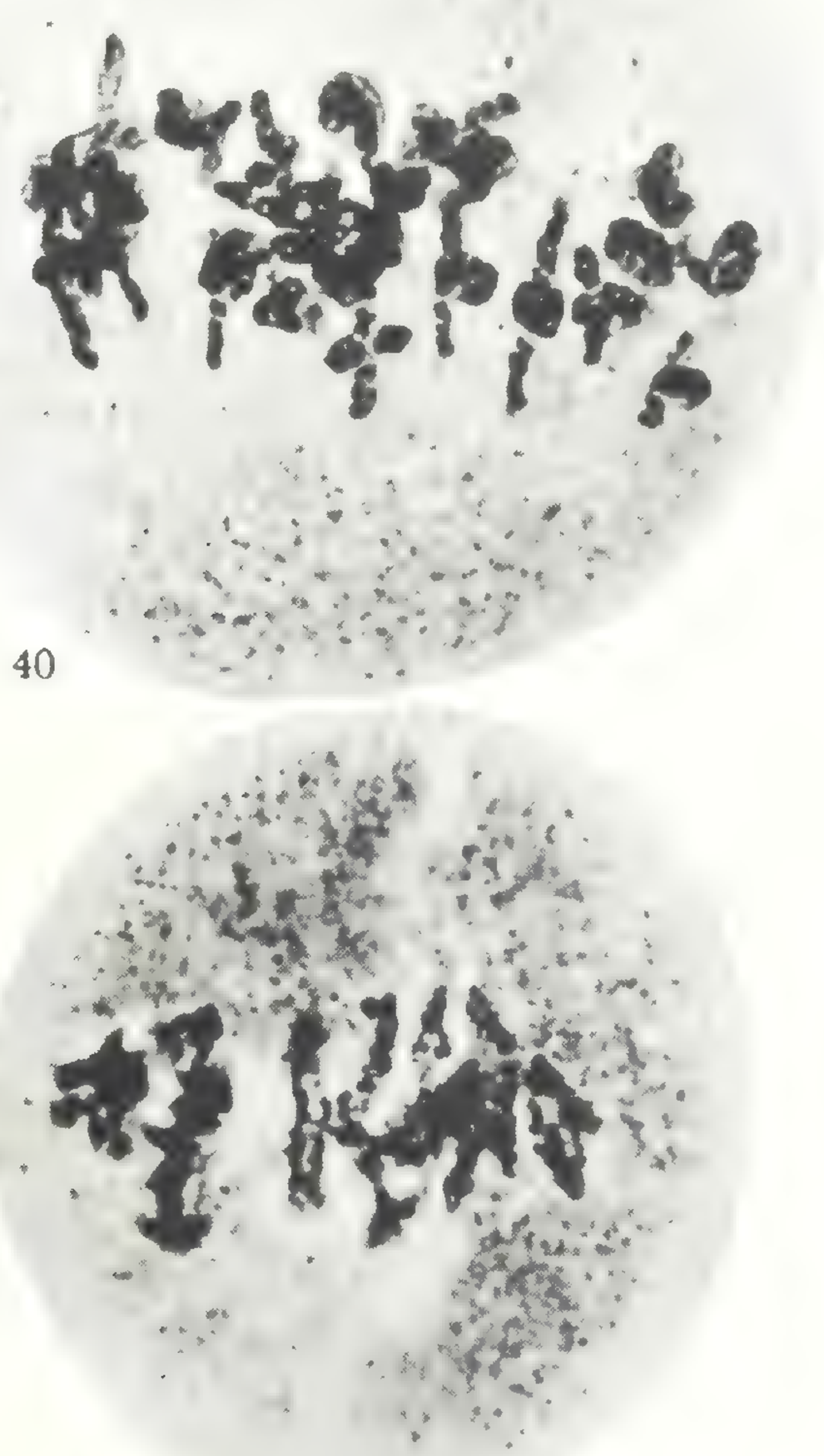

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Conifer Chromosomes

THE HELIOTYPE CORP. BOSTON

Plate 77.

Somatic chromosomes from endosperms.

Fig. 19. *Picea Abies.* Metaphase. 12 chromosomes.
Fig. 20. *Tsuga canadensis.* Late anaphase. 12 chromosomes.
Fig. 21. *Pseudotsuga taxifolia.* Metaphase. 13 chromosomes.
Fig. 22. *Abies cephalonica.* Metaphase. 12 chromosomes.
Fig. 23. *Abies concolor.* Metaphase. 12 chromosomes.
Fig. 24. *Cryptomeria japonica.* Late anaphase. 11 chromosomes.
Fig. 25. *Thuja occidentalis.* Late anaphase. 11 chromosomes.
Fig. 26. *Thuja orientalis.* Metaphase. 11 chromosomes.
Fig. 27. *Thuja plicata.* Metaphase. 11 chromosomes.
Fig. 28. *Juniperus virginiana.* Metaphase. 11 chromosomes.
Fig. 29. *Juniperus rigida.* Metaphase. 11 chromosomes.
Fig. 30. *Chamaecyparis Lawsoniana.* Metaphase. 11 chromosomes.

Plate 78.

Meiotic divisions in *Picea Abies.* Photographs of aceto-carmine preparations.

Figs. 31-36. Stages of meiosis from pachytene to tetrad formation.

Plate 79.

Meiosis in Conifer species. Photographs from aceto-carmine preparations.

Fig. 37. *Larix Kaempferi.* Diakinesis.
Fig. 38. *Pinus nigra.* Diakinesis.
Fig. 39. *Tsuga caroliniana.* Metaphase.
Fig. 40. *Pseudolarix amabilis.* Metaphase.
Fig. 41. *Cedrus libanotica.* Metaphase.
Fig. 42. *Abies cephalonica.* Metaphase.

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CHROMOSOME NUMBER AND RELATIONSHIP IN THE MAGNOLIALES

THOMAS W. WHITAKER

With plate 80 and four text figures

BECAUSE OF THEIR important phylogenetic position, the Magnoliales offer a suggestive group for cytological investigation. It is the purpose of the present paper to report the results of a cytological survey of several members of the Magnoliales with a discussion of the bearing of these results on taxonomic and phylogenetic relationships.[1]

The Magnoliales, according to Hutchinson (1921), may be separated into the following families: Magnoliaceae, Winteraceae, Schisandraceae, Himantandraceae, Lactoridaceae, Trochodendraceae, and Cercidiphyllaceae. In the collections of the Arnold Arboretum members of the Magnoliaceae, Schisandraceae, Trochodendraceae, and Cercidiphyllaceae are available for study. Through the coöperation of the Royal Botanic Garden, Kew, and the John Innes Horticultural Institution, Merton, England, it has been possible to extend the range of the investigation to include all the families in the order, with the exception of representatives of the Himantandraceae and Lactoridaceae.

Table I gives the chromosome number of the species studied. The observations on the meiotic divisions were made from aceto-carmine and permanent smear preparations of pollen mother cells. The aceto-carmine preparations were found to be superior for the study of chromosome number and chiasma frequency. The counts of *Illicium anisatum*, *I. floridanum* and *Drimys Winteri* were secured from root tip material by the use of the smear-maceration method followed by staining with aceto-carmine. The remaining somatic counts were secured from root tips by use of the usual permanent preparations of sectioned material.

[1]The work reported in the present paper has been furthered through the coöperation of a number of investigators at this and other institutions. Thanks are due to the officials of the Royal Botanic Garden, Kew, and of the John Innes Horticultural Institution, especially to W. J. C. Lawrence, Curator, and Mr. L. La Cour of the latter institution, who provided the material of *Trochodendron aralioides*, *Illicium religiosum*, *Tetracentron sinense*, and *Kadsura japonica*. Professor I. W. Bailey of the Arnold Arboretum has kindly furnished the data regarding the anatomy of the group studied. Professor Karl Sax of the Arnold Arboretum suggested the investigation and has very generously provided the drawings and information regarding *Cercidiphyllum* and *Euptelea*.

TABLE I

CHROMOSOME NUMBERS IN THE MAGNOLIALES

	Chromosome No. (n)	(2n)
MAGNOLIACEAE		
Magnolia virginiana L.		19
Magnolia tripetala L.		19
Magnolia Fraseri Walt.		19
Magnolia acuminata L.		38
Magnolia acuminata var. *cordata* Sarg.		38
Magnolia liliflora Desrouss		38
Magnolia Soulangeana Soul.		38
Magnolia Soulangeana var. *Brozzonii*		38
Magnolia Soulangeana var. *Candolleana*		38
Liriodendron Tulipifera L.		19
WINTERACEAE		
Illicium anisatum L.		28
Illicium religiosum Sieb. & Zucc.		28
Illicium floridanum Ellis		28
Drimys Winteri Forst.		±76 (4 × 19)
SCHISANDRACEAE		
Schisandra sphenanthera Rehd. & Wils.		14
Kadsura japonica L.		28
TROCHODENDRACEAE		
Trochodendron aralioides Sieb. & Zucc.		38
Euptelea polyandra Sieb. & Zucc.		14
CERCIDIPHYLLACEAE		
Cercidiphyllum japonicum Sieb. & Zucc.		19
Tetracentron sinense Oliv.[1]		38

In the genus *Magnolia,* the forms examined were either diploids or tetraploids (Pl. 80, figs. 1, 2, 5, 7). Morinaga, et al. (1929) have reported the haploid number of *M. grandiflora* as being 56—57. The basic number in *Magnolia* is 19. It therefore appears likely that *M. grandiflora* is a hexaploid.

Both diploid and tetraploid species were found among the Magnolias which are indigenous to North America. *Magnolia liliflora,* the only Chinese species which has been studied, has 38 chromosomes. Ishikawa (1916) has given the number of one of the Chinese species of

[1]*Tetracentron* has been omitted by Hutchinson (1926) in his treatment of the Magnoliales. Since it has generally been considered as belonging to this group, it has been included in this study.

Magnolia (*M. kobus*) as 19. This indicates that there are diploid and tetraploid representatives of the genus in both North America and Asia.

Magnolia Soulangeana is a supposed hybrid resulting from the cross *M. denudata* × *M. liliflora* (Rehder, 1927). The three forms of this hybrid that were examined all proved to be tetraploids (see Table I & Fig. 8). Since *M. liliflora* was found to be a tetraploid (Fig. 11), it is probable that the other parental species, *M. denudata*, is also a tetraploid. However, the chromosome count of this species was not secured. The meiotic divisions in the forms of *M. Soulangeana* that were examined show a slight amount of irregularity. There is also considerable pollen sterility, averaging about 25% in each of the three forms studied. In the parent species, *M. denudata* and *M. liliflora*, only 7% of the pollen is sterile.

Liriodendron Tulipifera has 19 pairs of chromosomes (Fig. 4). It is taxonomically closely allied to the Magnolias. This position is well supported by the cytological evidence. The chromosome size, shape, and chiasma frequency are similar to those of the Magnolias.

The chromosome constitution of the different species of *Magnolia* do not exhibit any detectable variation. The chromosomes are all short rods of equal length, showing up very clearly at metaphase. The nature of the material has made possible the determination of chiasma frequency at early metaphase. This has been done for *Liriodendron Tulipifera* and a few representative species of *Magnolia*.

The results of this study brought out the following points:

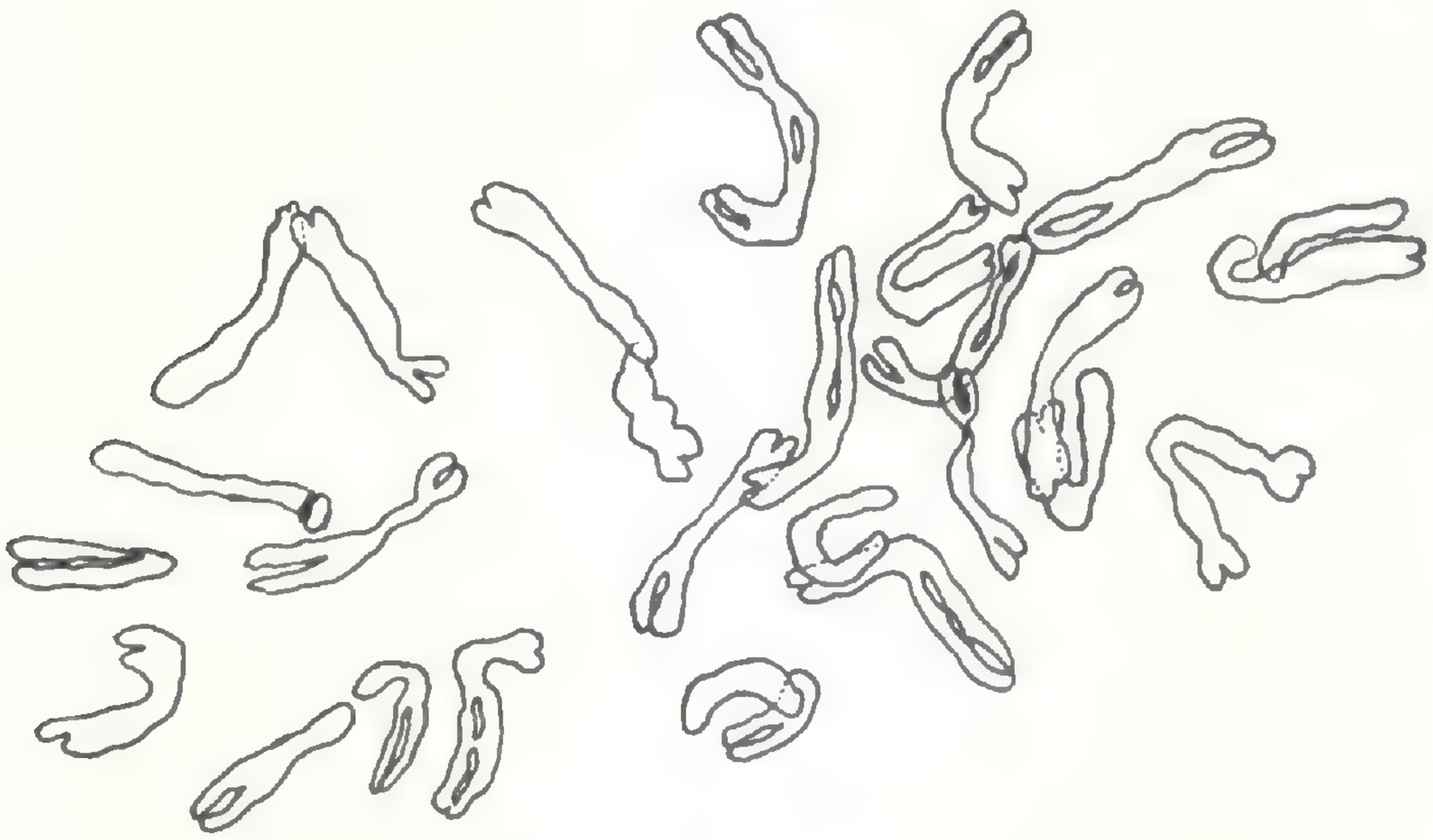
1. There was no significant difference in the chiasma frequency per bivalent between any of the species of *Magnolia* examined. Two diploid species (*M. virginiana* and *M. tripetala*), two tetraploid species (*M. acuminata* var. *cordata* and *M. liliflora*), and the species hybrid, (*M. Soulangeana* var. *Brozzonii*) were investigated. The chiasma frequency in the above species ranged from 1.06 in *M. tripetala* to 1.17 in *M. liliflora*. A difference of this degree can scarcely be considered significant. The chiasma frequency of *Liriodendron Tulipifera* was 1.14, thus coming within the same range as that of the *Magnolia* species.

2. By far the greater proportion of the bivalents are paired as rods. There are usually 1—3 rings and, in the tetraploids, one or two multivalent associations. The chiasmata are invariably terminal at this stage (early metaphase).

It was thought that a study of the pollen grains of the diploid and tetraploid Magnolias might show size differences which could be used as a diagnostic character in predicting whether a particular species was

a diploid or a tetraploid. It was found, however, that the pollen grains of both diploid and tetraploid species are all very similar in size, shape, and wall sculpturing. The pollen grains of *Liriodendron Tulipifera* bear certain resemblances to those of the Magnolias, although the pollen of this species appears to have a much thicker wall, with deeper, heavier sculpturing.

Three species of *Illicium* have been examined, all of which proved to be diploids with 28 somatic chromosomes (Text fig. 1). The chromosomes of the three species that have been examined are remarkably



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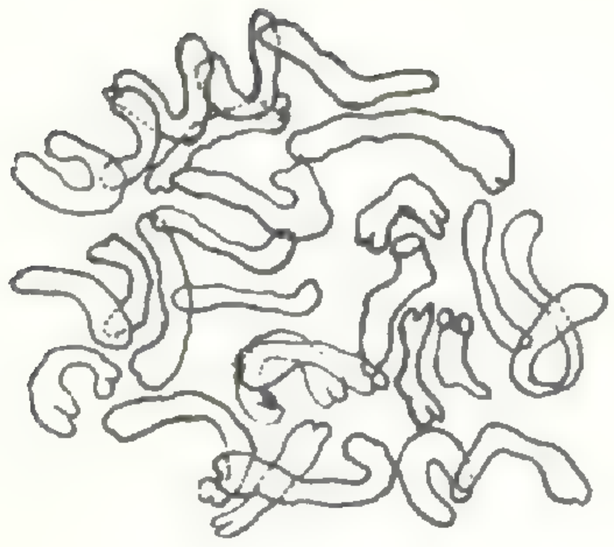

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FIGURE 1. ILLICIUM FLORIDANUM .....28 chromosomes.
FIGURE 2. KADSURA JAPONICA .....28 chromosomes.
FIGURE 3. TROCHODENDRON ARALIOIDES .....38 chromosomes.
FIGURE 4. TETRACENTRON SINENSE .....38 chromosomes.

FIGURE 1. has been drawn from a root tip smear. The remainder are from permanent preparations of root tip material. × 2100.

alike and are considerably larger than those of *Drimys* and other members of the group having 19 as the basic number. Cytologically, *Illicium* is clearly distinct and bears no resemblance to either the members of the Magnoliaceae or *Drimys*, supposedly its closest allies.

Morinaga, et al. (1929) have listed the reduced chromosome number of *Illicium anisatum* as 14. The figures given by these workers, of the chromosomes during metaphase of the first division compare favorably in size and shape with those of *Schisandra sphenanthera* (Pl. 80, fig. 10).

Drimys Winteri has about 76 chromosomes in somatic cells. Strasburger (1905) has stated there are about 36 pairs of chromosomes in this species. Because of the large number of chromosomes and their small size, it is difficult to make absolutely certain of the number. However, it is undoubtedly between 72 and 76, with greater likelihood of the latter figure's being correct.

In *Schisandra sphenanthera* the haploid chromosome number is 14 (Pl. 80, fig. 10). The chromosome complement of this species has no affinity with that of the Magnolias or *Liriodendron*. The chromosomes are larger than those found in the Magnoliaceae, and there is a tendency toward irregularity in the time of division,—i. e., precocious division of one or two bivalents. This does not seem to affect the percentage of good pollen (94%).

Kadsura is a genus of climbing vines, closely allied to *Schisandra*. *Kadsura japonica*, the only species which has been examined, has 28 chromosomes (Text fig. 2). The counts were made from permanent root tip preparations. The chromosomes of this species resemble those of *Schisandra* and other representatives of the 14 chromosome group.

Trochodendron aralioides has a somatic chromosome number of 38 (Text fig. 3). Cytologically, its relations are with *Magnolia* and *Liriodendron* rather than with *Euptelea*, with which it has been placed by Hutchinson (1921).

Euptelea polyandra has 14 pairs of chromosomes (Pl. 80, fig. 9). Some secondary pairing and irregularity in the time of division, similar to that found in *Schisandra sphenanthera*, has been noted in this species.

Cercidiphyllum japonicum, the representative of the monotypic family Cercidiphyllaceae has 19 chromosomes (Pl. 80, fig. 3). The figure indicates that the chromosome size and shape are of the same general nature as those of *Liriodendron* and *Magnolia*. The chiasma frequency corresponds closely to that found in these two genera.

Tetracentron sinense has 38 chromosomes in the root tip cells (Text fig. 4). Judging by its cytological characteristics, this genus is allied to the Magnoliales. Its chromosome number definitely places it with *Magnolia, Liriodendron, Trochodendron,* etc.

DISCUSSION

Engler and Prantl's system (Die natürlichen Pflanzenfamilien) has been criticized on the ground that the Amentiferae are regarded as a more primitive group than such polypetalous families as Magnoliaceae and Ranunculaceae. From morphological evidence, this criticism seems justified. Maneval (1914) has compiled a list of characters which are generally conceded to be more or less indicative of a primitive condition. These characters are all found in the Magnoliaceae. Sinnott (1914) has used nodal anatomy as a means of indicating phylogenetic relationships. He states: "The Magnoliaceae are perhaps more variable in nodal structure than any other family of dicotyledons." The Magnoliaceae, as used here, include, in addition to the Magnolias and *Liriodendron,* the Schisandraceae and Winteraceae of Hutchinson (1926). This variability in nodal structure is precisely what one would expect and what is actually found among primitive types.

If one accepts the customary thesis that the Magnoliales are a primitive group of dicotelydons, one would expect to find, among the families of this order, several lines of cytological development. Thus far the evidence from chromosome numbers and other cytological features indicates that there are at least two such lines. The first comprises *Magnolia, Liriodendron, Drimys, Trochodendron, Tetracentron,* and *Cercidiphyllum.* In this group the chromosomes are characteristically small, short rods with a basic number of 19 pairs. In the second group occur *Euptelea, Schisandra, Kadsura* and *Illicium,* with 14 as the basic chromosome number. The chromosome size, shape, and configurations found in this group bear considerable resemblance to one another.

In Table II an attempt has been made to compare chromosome number, type of nodal anatomy, and several other anatomical features in the genera under investigation. Perhaps the most important point brought out by this table is the correlation existing between chromosome number and type of nodal anatomy. In Group I are found genera with two types of nodal anatomy: (a) 3 traces from 3 gaps (*Tetracentron, Drimys, Cercidiphyllum*); (b) ∞ traces from ∞ gaps (*Trochodendron, Magnolia, Liriodendron*). The second cate-

TABLE II

A COMPARISON OF CHROMOSOME NUMBER, NODAL ANATOMY, ETC. IN GENERA OF THE MAGNOLIALES[1]

		genus	leaf venation	vessels present or absent	vessels scalariform or porous	tracheids
GROUP I basic chromosome number—19	Type of nodal anatomy *3 traces from 3 gaps*	*Tetracentron*	palmate	absent		scalariform
		Drimys	pinnate	absent		rarely scalariform
		Cercidiphyllum	palmate	present	scalariform	
	Type of nodal anatomy *∞ traces from ∞ gaps*	*Trochodendron*	pinnate	absent		scalariform
		Magnolia	pinnate	present	scalariform or porous	
		Liriodendron	pinnate	present	scalariform or porous	
GROUP II basic chromosome number—14	Type of nodal anatomy *1 trace from 1 gap*	*Illicium*	pinnate	present	scalariform	
		Schisandra	pinnate	present	scalariform or porous	
		Kadsura	pinnate	present	scalariform or porous	
	Type of nodal anatomy *3-7 traces from 1 gap*	*Euptelea*	pinnate	present	scalariform	

[1]Morphological data supplied by I. W. Bailey.

gory is essentially a multiplication of the fundamental condition of 3 traces from 3 gaps. In the 14-chromosome group two types of nodal anatomy are found; (a) 1 trace from one gap (*Illicium, Kadsura, Schisandra*); (b) 3—7 traces from 1 gap (*Euptelea*). The latter condition simply represents a multiplication in the number of traces of the basic condition of 1 trace from 1 gap.

The value of nodal anatomy as an aid in classification of angiosperms and as a means of detecting phylogenetic relationships, has been fully discussed by Sinnott (1914). It is sufficient, for the present purpose, to note that it is extremely constant within most families and is of considerable value in determining broad lines of relationship. The fact that there is a close agreement between chromosome number and nodal anatomy is reasonably strong evidence that the genera placed together in Table II form a natural grouping which should be considered in any future classification of this group.

It may be well to point out, at this juncture, the significance attached to a basic number of 19 chromosomes. This is an unusual number in the plant kingdom and is very rarely met. It has not been reported in any of the families which may be considered as related to the Magnoliales. If the chromosome number had been 12 or some other number of common occurrence, it would not carry nearly the weight which it does in the present case. It is difficult to account for the occurrence of this unusual chromosome number in Group I unless we assume that the genera composing this group are made up of closely related forms.

McLaughlin (1933) has recently made a very thorough study of the systematic anatomy of the woods of the Magnoliales. In his suggested classification of the group he has eliminated *Cercidiphyllum, Euptelea* and *Illicium* by placing them in the Hamamelidales rather than in the Hamamelidaceae. Cytologically, there is no evidence to justify this step. Professor Sax informs me that the Hamamelidaceae have 12 rather characteristically small, short chromosomes (unpublished data). *Illicium* and *Euptelea*, with 14 chromosomes, and *Cercidiphyllum*, with 19 chromosomes, would not fit into this proposed classification.

The placing of *Drimys* and *Illicium* in the same family, as proposed by Hutchinson (1921), can hardly be sanctioned in view of the present evidence. These two genera are very dissimilar cytologically. The same criticism can be made in regard to placing *Trochodendron* and *Euptelea* in the same family, as this author has suggested (Hutchinson, 1921).

SUMMARY

The evidence shows that in the Magnoliales thus far investigated there are two lines of cytological development. Group I has a basic chromosome number of 19. The chromosomes of this group are characteristically small, short rods. In this group the following genera occur: *Magnolia, Liriodendron, Cercidiphyllum, Drimys, Trochodendron,* and *Tetracentron.* Group II has a basic number of 14 chromosomes. In this group the chromosomes are much larger than those of Group I. The following genera occur in Group II: *Illicium, Schisandra, Kadsura,* and *Euptelea.*

There seems to be a correlation between basic chromosome number and type of nodal anatomy. Group I is characterized by the trilacunar condition and a multiplication of this condition. Group II is characterized by the unilacunar condition and a modification of this condition.

Some of the more recent systems of classification of the Magnoliales have been criticised, and it has been pointed out where certain revisions would bring about a more natural system of classification.

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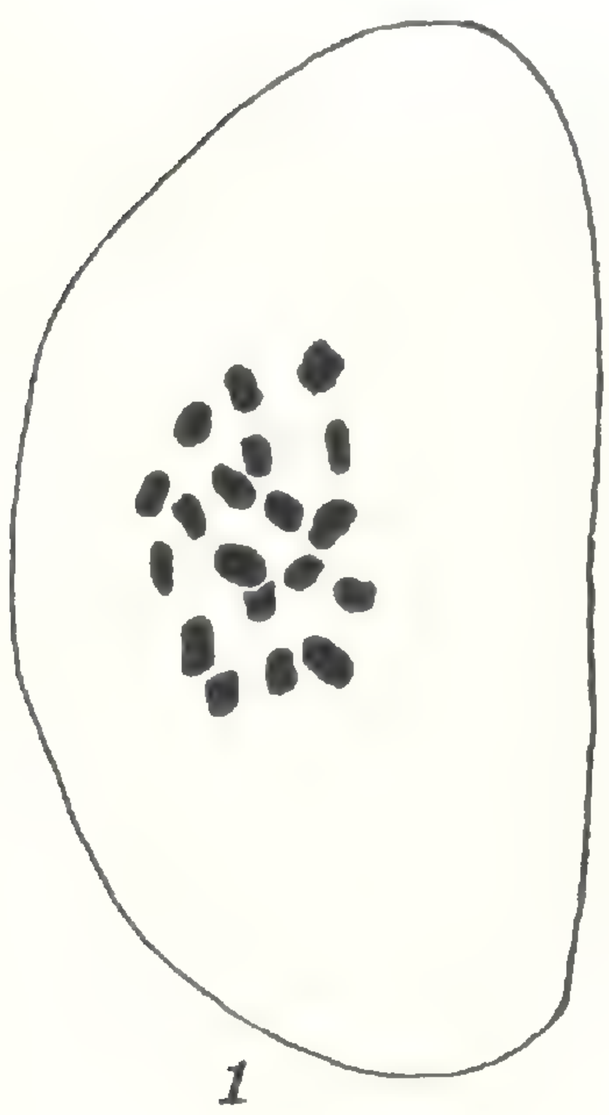

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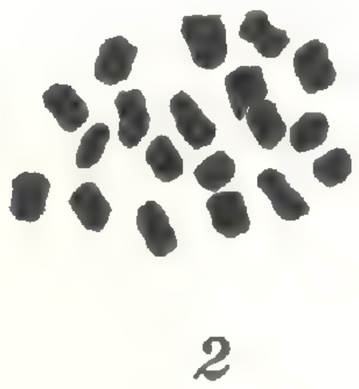

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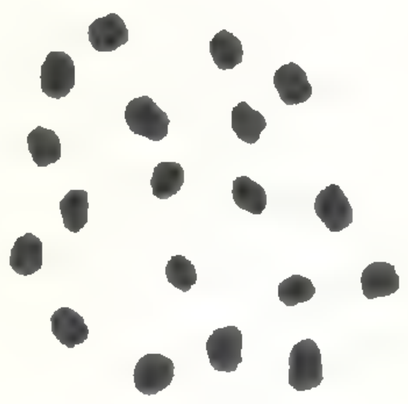

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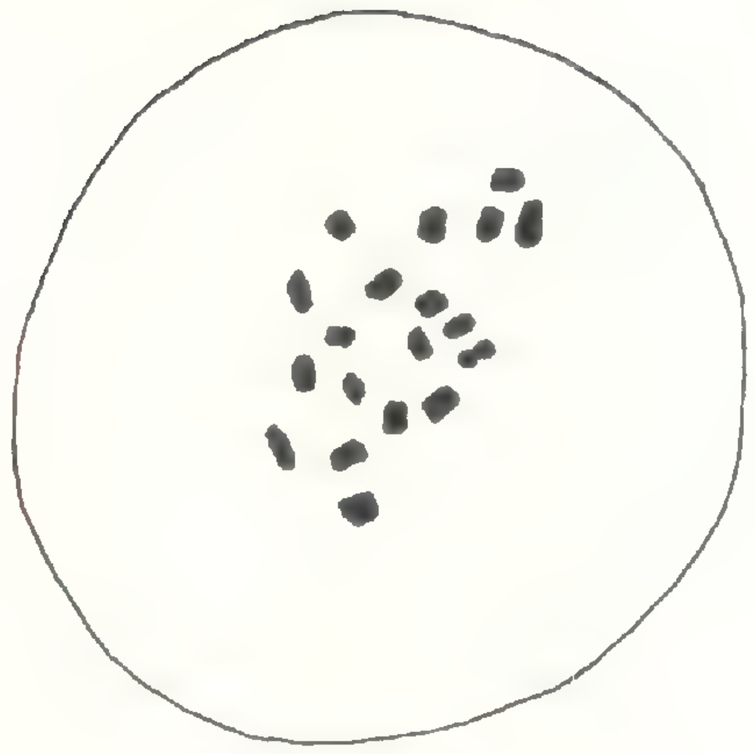

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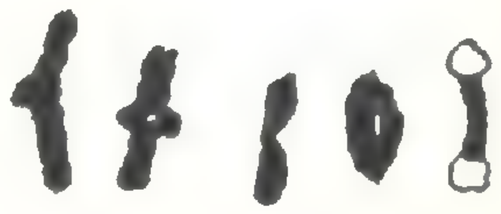

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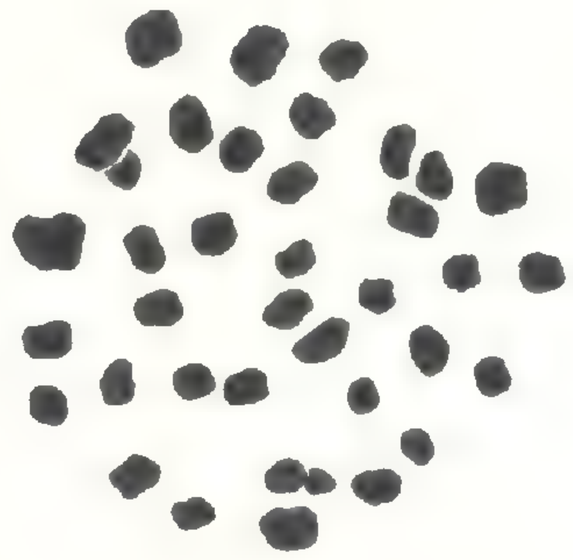

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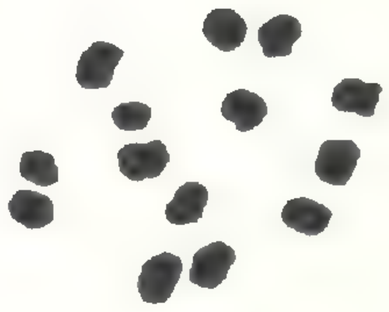

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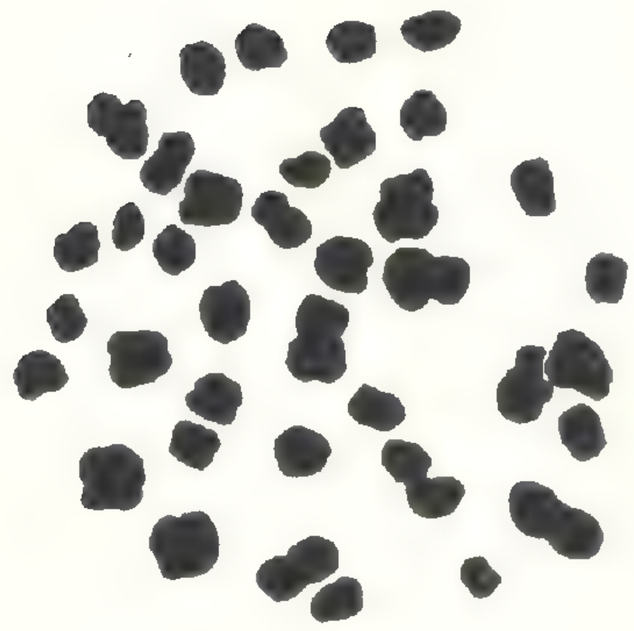

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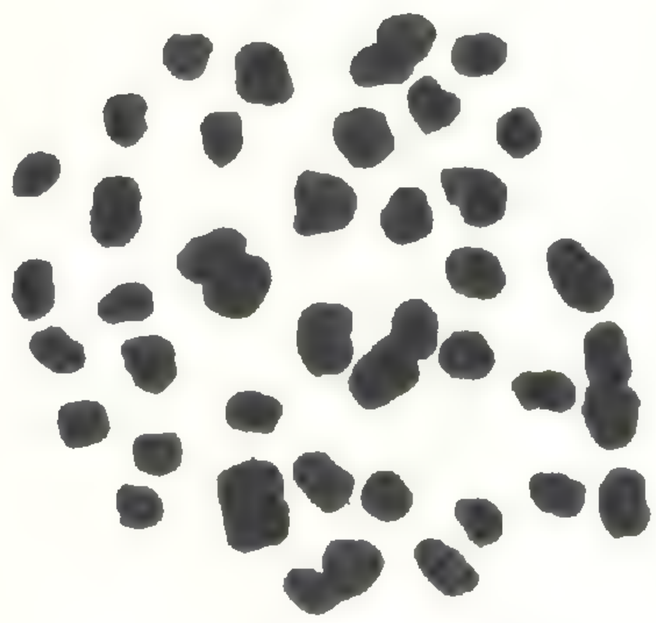

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DESCRIPTION OF PLATE 80

Chromosome numbers in the Magnoliales. The drawings are from aceto-carmine and permanent smears of pollen mother cells. Magnification about 2100.

Fig. 1. *Magnolia tripetala* ..... II M.
Fig. 2. *Magnolia virginiana* ..... I M.
Fig. 3. *Cercidiphyllum japonicum* ..... I M.
Fig. 4. *Liriodendron Tulipifera* ..... I M.
Fig. 5. *Magnolia Fraseri* ..... I M.
Fig. 6. Types of configurations found in *Liriodendron Tulipifera.*
Fig. 7. *Magnolia acuminata* ..... I M.
Fig. 8. *Magnolia Soulangeana* var. *Brozzonii* ..... I M.
Fig. 9. *Euptelea polyandra* ..... I M.
Fig. 10. *Schisandra sphenanthera* ..... I M.
Fig. 11. *Magnolia liliflora* ..... I M.

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CHROMOSOME NUMBER IN ACER AND STAPHYLEA

ROBERT C. FOSTER

With plate 81

INTRODUCTION

ACCORDING TO HUTCHINSON (1926) *Acer* is a large genus distributed widely throughout the northern temperate zone in North America, Europe, western Asia, China, Japan, and is found also in northern Africa. This large group of approximately 115 species is divided into at least 14 subgroups. Eight of these subgroups are represented in this study by 13 species and varieties. Two more subgroups, represented by one species in each, have been studied by Taylor (1920). In all, 10 of the 14 subgroups and 19 species and varieties have been studied by various workers, affording a rather broad basis for conclusions.

Closely allied to the Aceraceae are the Staphyleaceae. In the genus *Staphylea* two species have been previously studied (Mottier, 1914, Winge, 1917). These two have been re-examined and two additional species studied in the present work.

MATERIALS

In the spring of 1933, branches bearing male flowers of *Acer* and *Staphylea* were brought into the laboratory and examined for reduction divisions in the pollen mother-cells and for microscope divisions. The reduction divisions in *Acer* apparently occur very early, when flower buds and anthers are extremely small. A similar condition is true of *Staphylea*. Out of a large amount of material, satisfactory stages were found in only 13 species and varieties of *Acer* and 4 species of *Staphylea*. All material examined for chromosome counts was studied from aceto-carmine smears prepared according to Belling's formula (Belling 1926).

In addition to chromosome counts, preparations of the fully developed pollen were made and examined for pollen sterility counts. These counts were made on 53 species and varieties of *Acer*. All material was gathered in the Arnold Arboretum.

RESULTS

The chromosome counts and the pollen sterility counts on the species of *Acer* examined cytologically are summarized in Table I. For com-

TABLE I.

Group		Species	n	2n	Sterility of pollen	Author	Distribution (According to Rehder 1927)
I.	Platanoidea	platanoides L.	13	26	1%	Meurman, 1933	Europe and Caucasus
		Miyabei Maxim.	13		5%		Japan
II.	Campestria	campestre L.	13		1-3%		Europe; Western Asia
III.	Saccharina	saccharum Marsh.	13		5%	Taylor, 1920	East Canada, south to Ala., Ga., Miss.
IV.	Spicata	pseudoplatanus L.	26	52	1%	Taylor, 1920	Europe, West Asia
		pseudoplatanus var. erythrocarpum Carr.	26		1-2%		
V.	Palmata	circinatum Pursh	13		10-50%		Brit. Columb. to Calif.
		palmatum Thunb. var. intermedium	13		20%		
		pseudo-sieboldianum Komar.	13		10%		Manchuria to Korea
VIII.	Indivisa	carpinifolium Sieb. & Zucc.		52		Taylor, 1920	Japan
IX.	Macrantha	rufinerve Sieb. & Zucc.	13		1-5%		Japan
		Tschonoskii Maxim.	13		2%		Japan
XII.	Rubra	rubrum L.	40			Darling, 1912	Newfoundland to Fla., west to Minn. and Texas
		"	36			{Taylor, 1920 {Mottier, 1914	
		"	±50	88-94		Taylor, 1920	
		"	68-75			" "	
		"	52		5%		
		saccharinum L.	26	52 & ±91		Taylor, 1920	Que. to Fla., west to Minn. & Okla. Blooms very late in fall
XIII.	Trifoliata	nikoense Maxim.	13				Japan, Central China
		griseum Pax.	13		1-5%		West China
		mandshuricum Maxim.	13				Manchuria, Korea
XIV.	Negundo	Negundo L.	13		20%	Taylor, 1920 Darling, 1909 Sinoto, 1929	New England, southward
		Negundo var. interius Sarg.	13		25%		Alberta & Saskatoon to Ariz. & New Mex.

pleteness, this table also includes counts made by other workers and a brief indication of the geographic distribution of these species, according to Rehder (1927). Without exception, the haploid chromosome numbers found in this study were 13 or multiples of 13.

Of *Staphylea,* four species were examined. The results are summarized in Table II.

TABLE II.

Species	n	Distribution
Staphylea bumalda DC	13	Japan
" pinnata L.	13	Central and southern Europe
" colchica Stev.	26	Caucasus
" trifolia L.	39	Eastern Canada and U. S.

Here, as in *Acer,* the haploid number is either 13 or a multiple of 13.

DISCUSSION

Of the 19 species and varieties of *Acer* which have been examined cytologically, 14 are diploids, with n = 13. Four are tetraploids, with n = 26; one of these, *A. carpinifolium* has had only its somatic number studied (Taylor, 1920). *A. rubrum* is an octoploid, with n = 52. The Maples, then, have 13 as a basic number and are, for the most part, diploids, but a polyploid series does exist.

Differing counts have been made by other workers. Cardiff (1906) found n = 11 in *A. platanoides,* a count corroborated by Taylor (1920) who found, however, 2n = 26 in somatic counts on seedlings of this species. Meurman (1933) finds n = 13 in this species, as did Darling (1923). One seedling which the former examined proved to be a triploid, 2n = 39, which he regards as either an autotriploid, or a hybrid between a diploid and a tetraploid (loc. cit. p. 159).

In *A. rubrum,* for which n = 52 was found, four other counts have been made by Darling (1912), Mottier (1914) and Taylor (1920). The last named worker, in one instance, made a meiotic count of n = ±50, which approximates the count made in the present study.

The varying counts made on *A. rubrum* make plausible Taylor's (1920) suggestion of races within this species, possessing different chromosome numbers. Such a condition is known in other species, such as *Musa sapientium* L. (Tischler, 1910, 1928).

The count of n = 13 in *A. Negundo* has been made by three different workers, Darling (1909), Taylor (1920) and Sinoto (1929), although Mottier (1914) found n = 12 or 14.

The chromosomes in *Acer* are quite small in size. In shape they

have been described as "elongated" (Mottier, 1914), "ovoid" (Taylor, 1920), and "irregularly polygonal" by Sinoto (1929) who denies the accuracy of the first two descriptions. All three characterizations, as a matter of fact, are correct, as can be seen from the accompanying figures. Elongated chromosomes are clearly shown in Fig. 6, and both ovoid and irregularly polygonal types in Figs. 2, 3, and 5. The apparent shape undoubtedly varies with the angle at which the mitotic figure is oriented with regard to the observer.

All the forms studied showed complete formation of bivalents. Even in polyploids like *A. rubrum,* no univalent or multivalent formations were seen. Meurman (1933) found a similar completeness of pairing in *A. platanoides,* as did Sinoto (1929) in *A. Negundo.* This situation is probably due to the fact that with a low chiasma frequency, there is little chance for the formation of multivalents. At metaphase I in the octoploids *A. rubrum* most of the bivalents have one chiasma and are in the form of rods; there are few if any rings. Meurman (loc. cit. Figs. 13 and 17) shows this to be true of *A. platanoides,* and the accompanying Figs. 1 and 7 of 1st metaphases, show this is the case in *A. Miyabei,* and *A. pseudoplatanus* var. *erythrocarpum.* From this it is apparent that the chiasma frequency is probably 1, although the occasional presence of a ring would raise it slightly above 1.

As a result of the regularly-formed bivalents, the chromosomes can be distributed regularly to the poles, even in polyploids like *A. rubrum.*

The phenomenon of secondary pairing noted by Meurman (1933) in *A. platanoides* is present in the species included in the present study. It is especially well shown in Figs. 6 and 8. Meurman (loc. cit. pp. 160 and 162) also found that 2 large pairs of bivalents showed secondary pairing at both the 1st and 2nd metaphases, but found no such pairing between other bivalents.

It was not found practicable to include a study of somatic chromosomes, but both Taylor (1920) and Meurman (1933) have published some details on this point. Taylor noted that the longest somatic chromosome studied was about 3 microns long, and the smallest, 1 micron long, with diameters of from 1/3 to 2/3 microns. Meurman found in the somatic chromosomes of *A. platanoides* lengths from 0.8—22 microns. Both writers, too, note the existence of regions of doubling of chromosome numbers in the root tips.

Pollen sterility counts were made on 53 species and varieties. Most of them showed a high percentage of good pollen. The species and varieties showing more than 80% good pollen were as follows: *A.*

platanoides L. and its varieties *cucullatum* Nichols, *Schwedleri* K. Koch, *Stollii* Spaeth, *dilaceratum* Dieck, *palmatifidum* Tausch, *columnare* Carr., *nanum* Nichols., *A. Miyabei* Maxim., *A. truncatum* Bge., *A. pictum* Thunb., *A. campestre* L., and its varieties *compactum* Schwerin and *postelense* Lauche, *A. saccharum* Marsh., *A. grandidentatum* Nutt., *A. pseudoplatanus* L. and its variety *erythrocarpum* Carr., *A. Heldreichii* Orph. var. *macropterum* Vis., *A. Trautvetteri* Medwed., *A. ginnala* Maxim. and its var. *aidzuense* Franch., *A. tataricum* L., *A. palmatum* Thunb. and its varieties *atropurpureum* Nichols., *sanguineum* Lem., *septemlobum* K. Koch, *intermedium* Schwerin, *elegans* Koidz., *Hessei* Schwer., *sinuatum* Schwer. and *laciniatum* Schwer., *A. Sieboldianum* Miq., *A. pseudo-Sieboldianum* Komar. and its variety *ambiguum* Nakai, *A. pennsylvanicum* L., *A. rufinerve* Sieb. & Zucc., *A. Tschonoskii* Maxim., *A. argutum* Maxim., *A. rubrum* L. and its variety *glaucum* Marsh., *A. griseum* Pax and *A. Negundo* L. and its varieties *pruinosum* Schwer. and *texanum* Pax.

Certain species display a high percentage of poor pollen, as follows:

A. Mayrii Schwerin	50%	bad
A. zoeschense Pax	50%	"
A. spicatum Lam.	55%	"
A. circatum Pursh	50%	"
A. tegmentosum Maxim.	100%	"
A. barbinerve Maxim.	90%	"
A. Negundo L., var. *interius* Sarg.	25%	"
A. Negundo, var. *nanum* Dieck.	50%	"

Such high percentages of pollen sterility, as contrasted with the lower figures for the others studied, indicate a possible hybrid origin of these species or structural hybridity. In the case of one species, *A. zoeschense*, Rehder (1926) indicates that it may be a hybrid between *A. campestre* L. and *A. Lobelii* Ten.

Little data on hybridization appear available. Rehder lists about 15 species hybrids, but they are usually between species in the same subgroup or in closely related subgroups. The widest cross noted was that between *A. opalus* Mill., var. *obtusatum* Henry in the *Campestria*, and *A. pennsylvanicum* L. in the group *Macrantha*.

Information on grafting supplied by William H. Judd of the Arnold Arboretum, indicates that *A. griseum* Pax and *A. parviflorum* Franch. & Sav. have not been used successfully in grafting with other species.

Ordinarily, too, a species can be used as stock or scion only with species closely related to it. Mr. Judd has found this to be particularly true of *A. platanoides*.

Chromosome conditions in *Staphylea* present a close parallel with those in *Acer*. The basic number is 13, but a tetraploid, *S. colchica*, n = 26, and a hexaploid, *S. trifolia*, n = 39, are found. In *S. pinnata* Winge (1917) found n = 12, but noted n = 13 in one cell. Mottier (1914) found n = ±36 in *S. trifolia*. Although the chromosomes are much larger than those of *Acer* it is difficult to determine their shape from polar views of meiotic metaphases. Like *Acer*, too, they appear to have a low chiasma frequency, and separation is quite regular. The secondary pairing is clearly shown in Figs. 10 & 11. As in *Acer*, this secondary pairing is between 2 bivalent chromosomes, and often makes an accurate count quite difficult.

The data thus presented show that in two genera belonging to different families there are identical basic chromosome numbers together with a similarity in polyploid series, secondary pairing, and low chiasma frequencies.

In *Acer* there is found a great differentiation of species in a highly polymorphic genus. Yet this process of species differentiation has taken place with no change in the basic chromosome number of the genus. Although only 1/6 of the known species of *Acer* have been studied, their distribution throughout the subgroups of the genus is sufficiently wide to make this statement reasonable. What is true of a genus is apparently true also of families. Despite a clear relationship, the Staphyleaceae are admittedly different from the Aceraceae morphologically, but the type genus, *Staphylea*, shows the same chromosome setup and the same general behavior, even to secondary pairing, which is found in *Acer*. It is true that there are differences in chromosome size between the two genera, but this is probably of no great significance. Such differences in chromosome size often exist between species within a genus or between varieties of the same species. Considered with other similarities, the common chromosome number and behavior may well indicate a common origin for these two closely related genera.

SUMMARY

1. Chromosome counts were made on the meiotic stages of thirteen species and varieties of *Acer* and four species of *Staphylea*. Thirteen was found to be the basic haploid number in each genus.

2. These counts, with those given by other workers show that most of the species are diploids. A polyploid series, however, is found in each genus.

3. The chromosomes of *Acer* are small, have a low chiasma frequency, behave regularly since no univalents or multivalents are present, and exhibit secondary pairing.

4. The chromosomes of *Staphylea* are larger than those of *Acer*, but show the same low chiasma frequency, regularity of behavior, and second pairing.

5. Pollen sterility counts were made on 53 species and varieties of *Acer*. Forty-five showed more than 80% good pollen. The remaining eight showed from 25-100% sterility.

6. Evidence from hybridization and grafting is briefly considered.

7. It is concluded that the cytological details afford evidence of a common origin for the Aceraceae and Staphyleaceae.

I wish to express my thanks to Dr. Karl Sax for his assistance in preparing material and drawings, especially those of *Staphylea*, and for his criticism of the manuscript, and to Dr. Haig Dermen who made all the pollen sterility counts.

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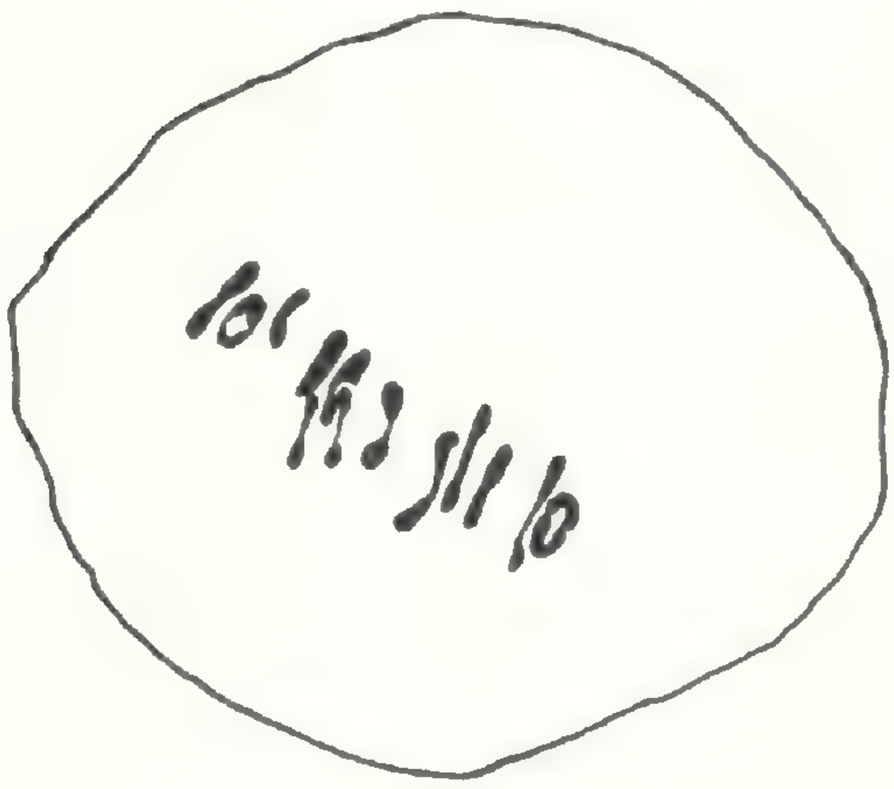

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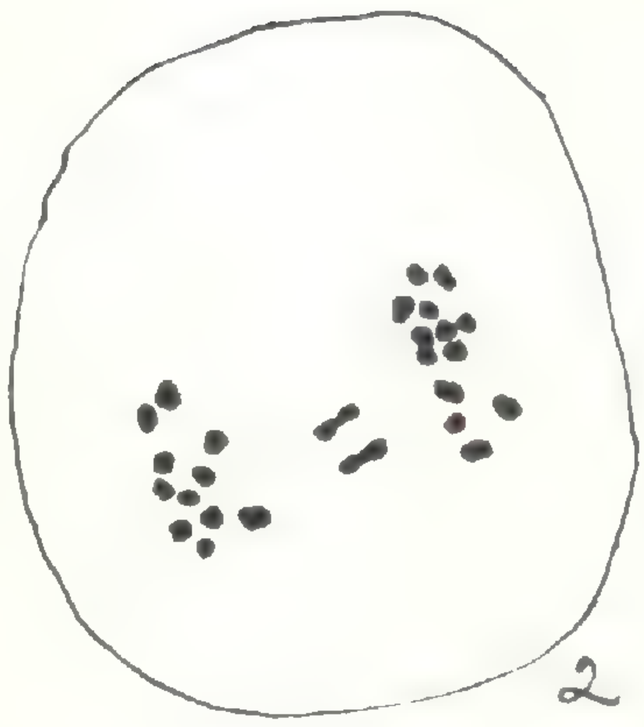

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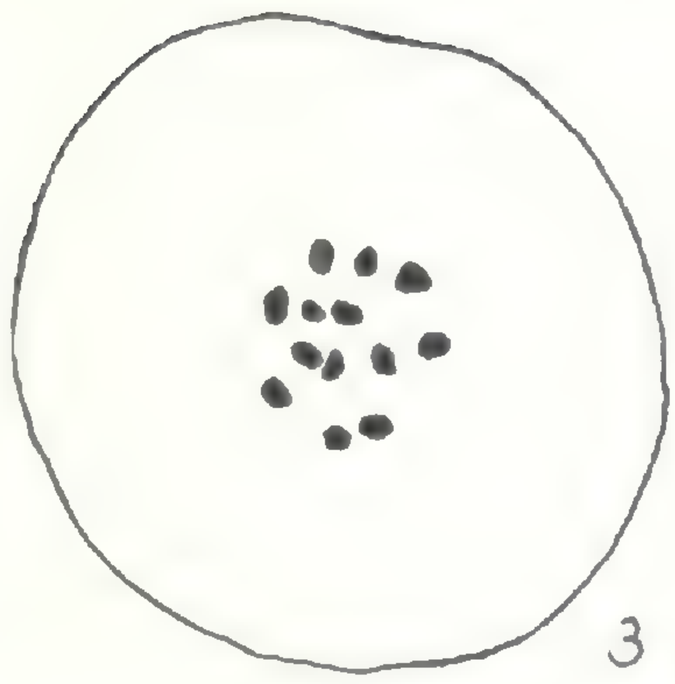

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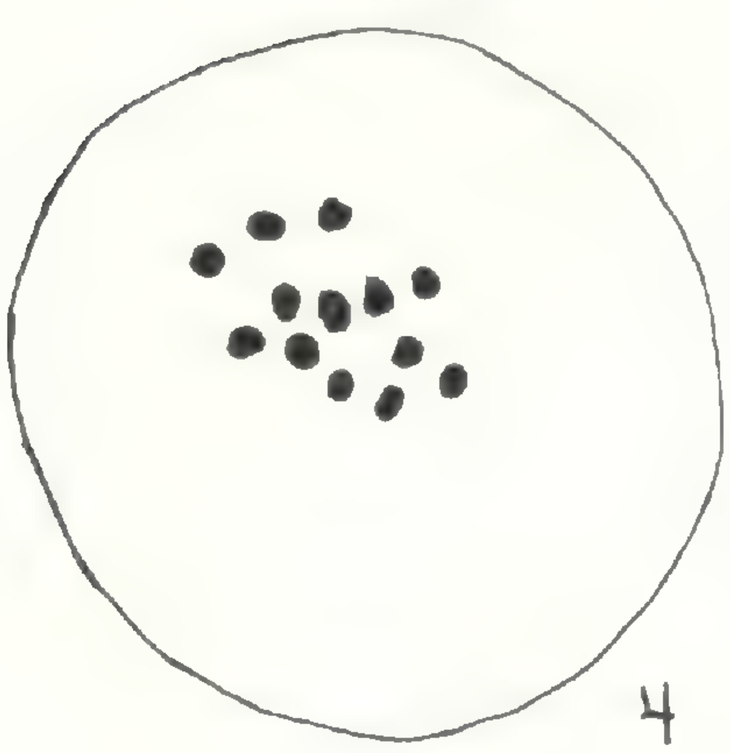

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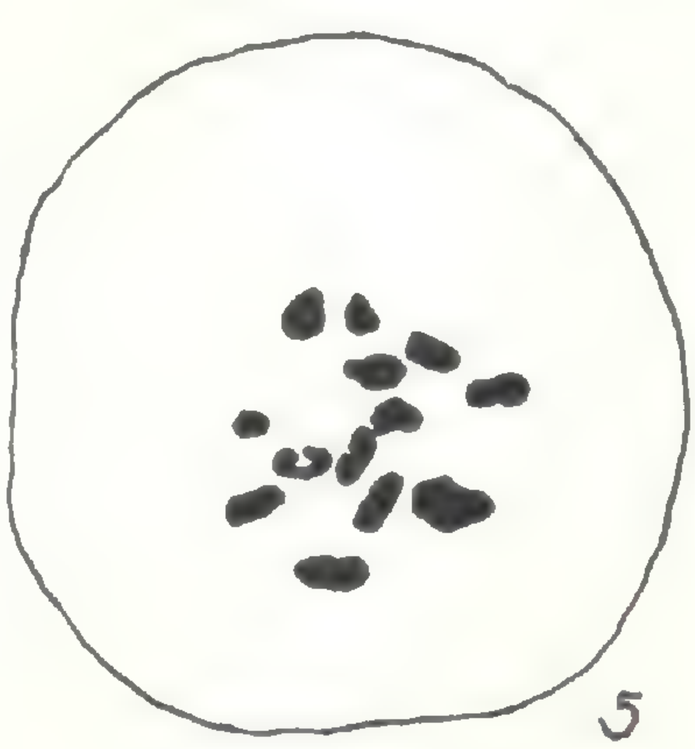

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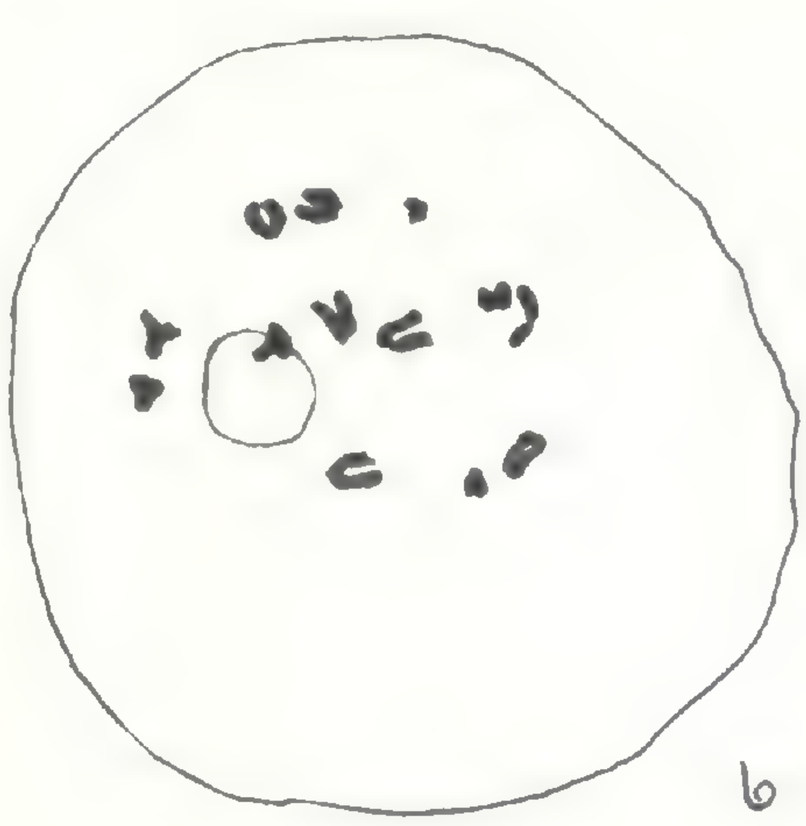

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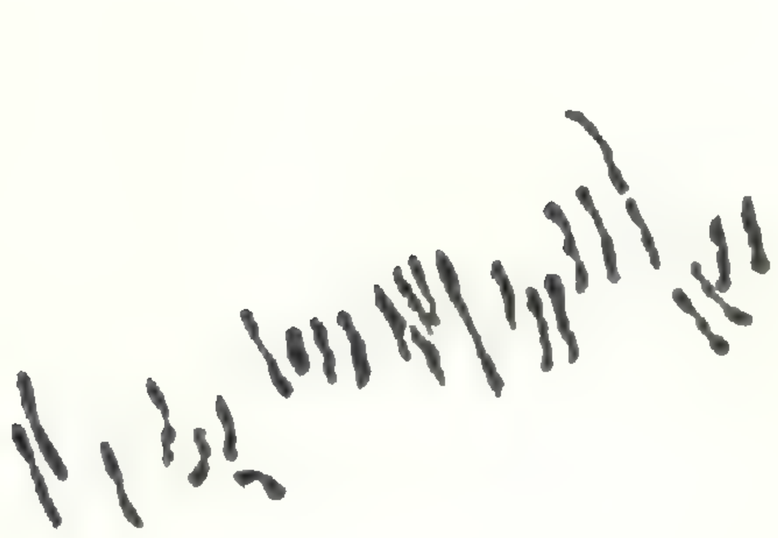

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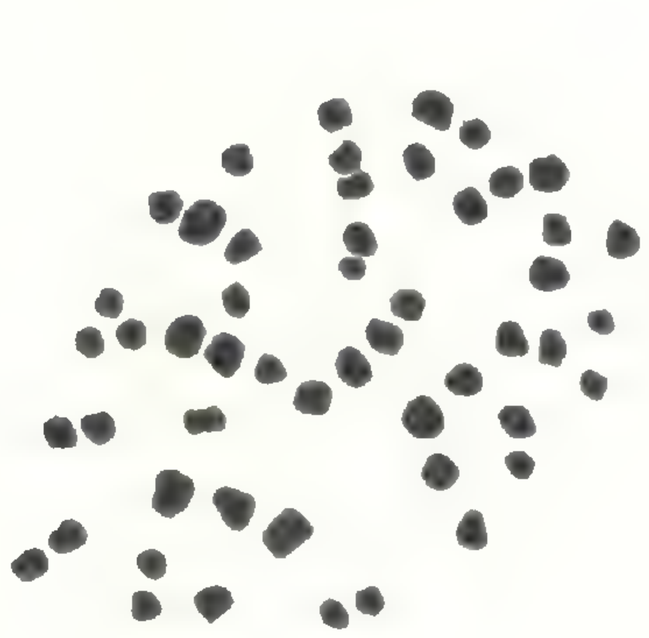

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CHROMOSOME NUMBER IN ACER AND STAPHYLEA

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EXPLANATION OF PLATE 81

The figures in the plate were drawn from aceto-carmine preparations of meiotic stages. The drawings were made with the aid of a camera lucida. Magnification ×1800, except Figure 8 which is about ×2450.

Fig. 1. *Acer Miyabei;* 1st metaphase, side view.
Fig. 2. *Acer circinatum;* 2nd telophase.
Fig. 3. *Acer pseudo-sieboldianum;* late diakinesis.
Fig. 4. *Acer Tschonoskii;* 1st metaphase, end view.
Fig. 5. *Acer griseum;* diakinesis.
Fig. 6. *Acer Negundo interius;* diakinesis.
Fig. 7. *Acer pseudoplatanus erythrocarpum;* 1st metaphase, side view.
Fig. 8. *Acer rubrum;* 1st metaphase, end view.
Fig. 9. *Staphylea pinnata;* 1st metaphase, end view.
Fig. 10. *Staphylea colchica;* 1st telophase.
Fig. 11. *Staphylea trifolia;* 1st metaphase, end view.

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STUDIES ON THE "PRECIPITIN REACTION" IN PLANTS
V. APPLICATION TO PLANT RELATIONSHIPS

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IN AN EARLIER PAPER of this series (2) the senior writer applied the "normal precipitin reaction," better designated by Foster and Avery (5) as the "precipitation reaction," to representatives of a number of families of plants for the purpose of determining whether or not the method is applicable in the study of plant relationships. In most of the material tested at that time there was an abundance of positive reactions, and these tended in general to harmonize with the findings of empirical taxonomy, although a uniformity of negative reactions in some groups limited the procedure to certain families in which well marked positive reactions were obtained. In 1933 Foster and Avery (5) applied a similar technique to the genus *Iris* with results which were satisfying and entirely consistent with those reported in the present series of studies. The purposes of the present paper are to provide additional data on the occurrence of the precipitation reaction to those already recorded, and then from a consideration of all data thus far obtained to analyze the significance of the precipitation reaction in a study of plant relationships with particular reference to the limitations and essential meaning of the reaction. The new groups tested in the present study are the "Amentiferae" and the Guttiferae. The following scheme gives the results of all precipitation tests thus far obtained including the results of the present study.

Family:	*Genera*:	*Tests of genera inter se*:	*Some positive tests obtained with*:	*Entirely negative tests with*:	*Reference*:
Iridaceae	Iris 30[1]	Numerous positive reactions correlated with systematic position.	Solanaceae[2] (CaC_2O_4) Oleaceae Caprifoliaceae Rosaceae "Amentiferae" Guttiferae	Saxifragaceae	Foster & Avery (5); Table IV.[3]

[1]The number refers to the number of species employed in the tests.

[2]Signifies that the only reactions observed were determined as due to calcium oxalate.

[3]Refers to tables in the present paper.

Family:	*Genera:*	*Tests of genera inter se:*	*Some positive tests obtained with:*	*Entirely negative tests with:*	*Reference:*
"Amentiferae"		Uniformly	Solanaceae	Rosaceae	Tables I,
Salicaceae:	Salix 1	negative	Oleaceae	Saxifragaceae	III, IV
Myricaceae:	Myrica 1		Iridaceae	Guttiferae	
Leitneriaceae:	Leitneria 1		Caprifoliaceae		
Juglandaceae:	Carya 1		(CaC_2O_4)		
Betulacaeae:	Alnus 15				
	Betula 16				
	Carpinus 6				
	Corylus 8				
	Ostryopsis 1				
	Ostrya 2				
Fagaceae:	Quercus 1				
Saxifragaceae:	Philadelphus 1	As in	Solanaceae	Iridaceae	Chester (2);
	Fendlera 1	Iridaceae	Rosaceae	"Amentiferae"	table IV
	Schizophragma 1		Platanaceae	Guttiferae	
	Hydrangea 1		Leguminosae		
	Jamesia 1		Oleaceae		
	Deutzia 1		(CaC_2O_4)		
	Itea 1		Caprifoliaceae		
	Ribes 1		(CaC_2O_4)		
Platanaceae:	Platanus 1		Solanaceae	Leguminosae	Chester (2)
			Rosaceae		
			Saxifragaceae		
			Oleaceae		
			(CaC_2O_4)		
			Caprifoliaceae		
			(CaC_2O_4)		
Rosaceae:					
Spiroideae:	Spiraea 1		Rosoideae	Pomoideae	Chester (2)
			Prunoideae	Platanaceae	
				Leguminosae	
				Saxifragaceae	
Pomoideae:	Cotoneaster 1	Generally	Iridaceae	Platanaceae	Chester (2);
	Stranvaesia 1	negative	Solanaceae	Leguminosae	table IV
	Chaenomeles 1		Rosoideae	Caprifoliaceae	
	Amelanchier 1		Prunoideae	"Amentiferae"	
	Pyracantha 1		Saxifragaceae	Guttiferae	
	Mespilus 1		Oleaceae		
	Crataegus 1		(CaC_2O_4)		

Family:	*Genera*:	*Tests of genera inter se*:	*Some positive tests obtained with*:	*Entirely negative tests with*:	*Reference*:
Pomoideae: (*continued*)	Sorbus 1 Aronia 1 Photinia 1 Malus 3 Pyrus 1				
Prunoideae:	Prunus 15 Osmaronia 1 Prinsepia 1 Maddenia 1	As in Iridaceae	Saxifragaceae Leguminosae Pomoideae Platanaceae Solanaceae Oleaceae (CaC_2O_4) Caprifoliaceae (CaC_2O_4)	Iridaceae "Amentiferae" Guttiferae	Chester (2); table IV
Leguminosae:	Robinia 1		Solanaceae Rosaceae Saxifragaceae Oleaceae (CaC_2O_4) Caprifoliaceae (CaC_2O_4)	Platanaceae	Chester (2)
Guttiferae:	Hypericum 10	Uniformly negative	Saxifragaceae Solanaceae Oleaceae Iridaceae Caprifoliaceae (CaC_2O_4) Rosaceae	"Amentiferae"	Tables II, III, IV
Oleaceae:	Syringa 2 Ligustrum 8 Fraxinus 1 Chionanthus 1 Forsythia 1	Uniformly negative except for calcium oxalate reaction	Solanaceae Iridaceae "Amentiferae" Guttiferae Rosaceae (CaC_2O_4) Saxifragaceae (CaC_2O_4) Caprifoliaceae (CaC_2O_4) Platanaceae (CaC_2O_4) Leguminosae (CaC_2O_4)		Chester (1); table IV

Family:	*Genera*:	*Tests of genera inter se*:	*Some positive tests obtained with*:	*Entirely negative tests with*:	*Reference*:
Solanaceae:	Lycopersicum 1 Salpiglossis 1 Cyphomandra 1 Browallia 1 Nicotiana 19 Capsicum 2 Solanum 5 Physalis 1 Petunia 1 Lycium 1 Datura 4 Atropa 1	As in Iridaceae	Oleaceae (CaC_2O_4) Iridaceae Rosaceae Saxifragaceae Caprifoliaceae Platanaceae Leguminosae "Amentiferae" Guttiferae		Kostoff (6); Chester & Whitaker (3); table IV
Caprifoliaceae:	Kolkwitzia 1 Symphoricarpus 1 Diervilla 1 Viburnum 1 Linnaea 1 Sambucus 1 Dipelta 1 Abelia 1 Lonicera 2	Uniformly negative	Solanaceae Iridaceae Rosaceae Guttiferae Oleaceae (CaC_2O_4) Saxifragaceae (CaC_2O_4) Platanaceae (CaC_2O_4) Leguminosae (CaC_2O_4) "Amentiferae" (CaC_2O_4)		Chester (2); table IV

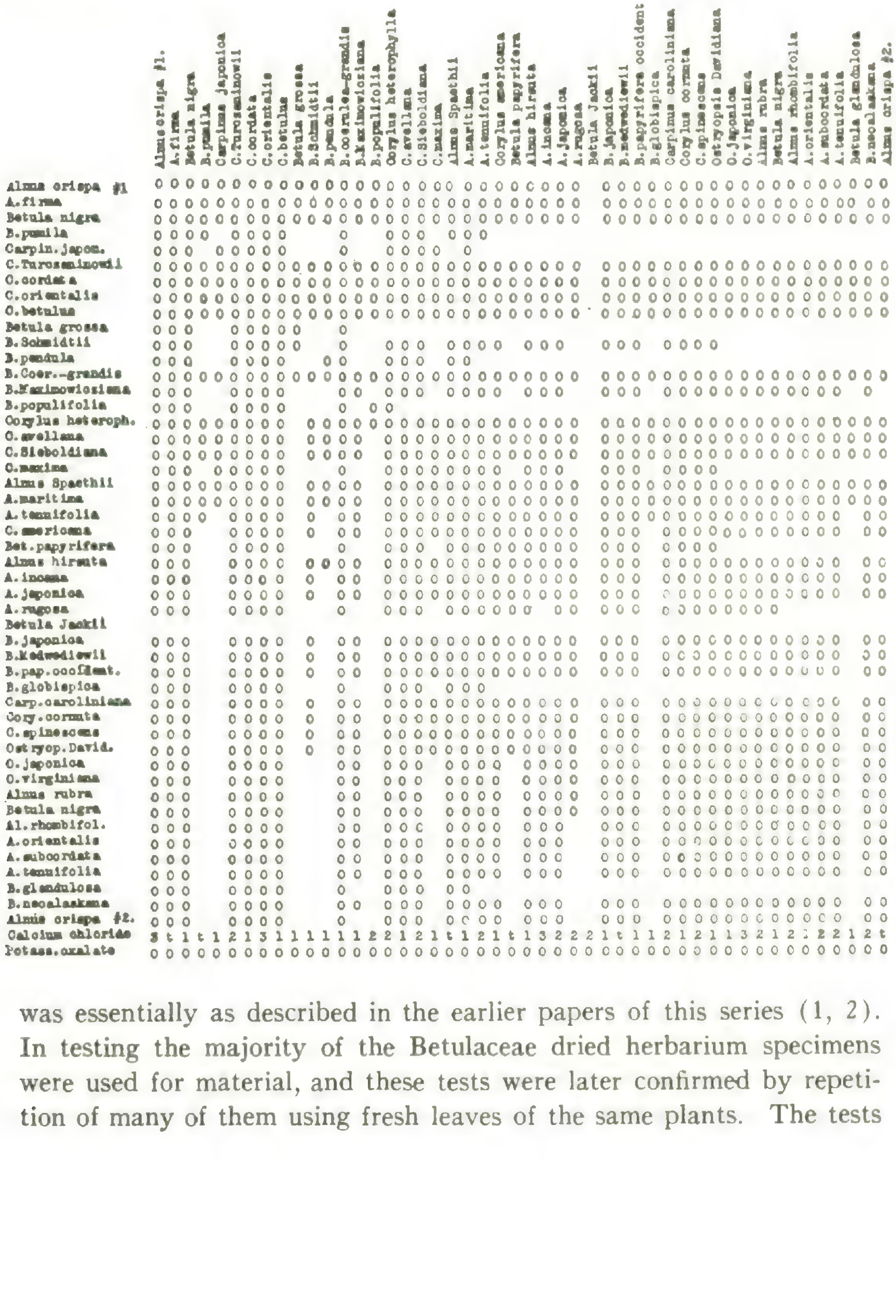
Thus up to the present fifteen families and approximately two hundred species of plants have been tested more or less extensively with regard to the precipitation reaction. Of these fifteen families, four (Solanaceae, Iridaceae, Saxifragaceae and Rosaceae-Prunoideae) have yielded among themselves significant positive results from the taxonomic standpoint. In five of the other eleven groups (Oleaceae, Rosaceae-Pomoideae, Caprifoliaceae, Guttiferae, "Amentiferae") fairly extensive tests within the groups have yielded wholly negative results, while in the remaining seven families the results thus far obtained are inadequate for sound conclusions because the number of species tested is too limited.

The present paper reports the results obtained in tests of the precipitation reactions of forty-five species of Betulaceae, one species each

of five other families of "Amentiferae," ten species of *Hypericum* (Guttiferae), and two species each of the Iridaceae, Solanaceae, Oleaceae, Rosaceae, Caprifoliaceae, and Saxifragaceae. The technique employed

TABLE I. PRECIPITATION REACTIONS IN THE BETULACEAE

"t"...trace; "1"...weak reaction; "2"...moderate reaction; "3"... strong reaction; "O"... no reaction. Experiments performed with dried herbarium material.



was essentially as described in the earlier papers of this series (1, 2). In testing the majority of the Betulaceae dried herbarium specimens were used for material, and these tests were later confirmed by repetition of many of them using fresh leaves of the same plants. The tests

of the other "Amentiferae" and other families mentioned above were performed with freshly collected leaves. The dried leaves were extracted for a few hours in ten times their weight of distilled water, and

TABLE II. PRECIPITATION REACTIONS IN HYPERICUM (GUTTIFERAE)

"t"...trace; "+"...weak reaction; "++"... moderate reaction; "+++"...strong reaction; "O"...no reaction. Experiments performed with fresh material.

	Hypericum Ascyron #1.	H.galioides	H.aureum	H.arnoldanum	H.Kalmianum	H.nudiflorum	H.lobocarpum	H.Buckleyi	H.boreale	H.perforatum	H.Ascyron #2.	Potass. oxalate	Calcium chloride
Hypericum Ascyron #1.	0	0	0	0	0	0	0	0	0	0	0	+	0
H.galioides	0	0	0	0	0	0	0	0	0	0	0	t	0
H.aureum	0	0	0	0	0	0	0	0	0	0	0	+	0
H.arnoldanum	0	0	0	0	0	0	0	0	0	0	0	t	0
H.Kalmianum	0	0	0	0	0	0	0	0	0	0	0	+	0
H.nudiflorum	0	0	0	0	0	0	0	0	0	0	0	t	0
H.lobocarpum	0	0	0	0	0	0	0	0	0	0	0	++	0
H.Buckleyi	0	0	0	0	0	0	0	0	0	0	0	0	0
H.boreale	0	0	0	0	0	0	0	0	0	0	0	t	0
H.perforatum	0	0	0	0	0	0	0	0	0	0	0	+	0
H.Ascyron #2.	0	0	0	0	0	0	0	0	0	0	0	+	0

the fresh leaves in three to four times their weight of distilled water. The results of these tests are given in Tables I, II, III, and IV below.

As is indicated in Table I an experiment involving forty-five species of the Betulaceae showed no positive results. These species represent every section of every genus of the family as recognized by Winkler (8)

with the exception of the section Cremastogyne in *Alnus*. In every case the material used was authenticated by herbarium specimens from which dried leaves were obtained for the tests. Through the kindness of Mr. Rehder of the Arnold Arboretum the determinations of these

TABLE III. PRECIPITATION REACTIONS IN THE "AMENTIFERAE"

Notations as in Table II. Experiments performed with fresh material.

	Ostryopsis	Ostrya virginiana	Carpinus cordata	Carpinus caroliniana	Corylus cornuta	Betula nigra	B.pumila	B.occidentalis	B.Maximowicziana	Alnus crispa	A.incana	Quercus alba	Leitneria floridana	Salix Matsudana	Carya alba	Myrica	Hypericum nudiflorum	Potass.oxalate.	Calcium chloride
Ostryopsis	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	++	0
Ostrya virginiana	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
Carpinus cordata	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
C.caroliniana	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
Corylus cornuta	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
Betula nigra	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
B.pumila	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
B.occidentalis	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
B.Maximowicziana	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0		+	0
Alnus crispa	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	++	0
A.incana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0
Quercus alba	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	++	0
Leitneria florid.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	++	0
Salix Matsudana											0		0			0	0	++	0
Carya alba	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	+	0
Myrica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0
Hypericum nudifl.	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	t	0

specimens were checked and the specific names are cited in accordance with his treatment of the family in his "Manual of Cultivated Trees and Shrubs" (7).

In view of the discrepancy observed in *Iris* by Foster and Avery (5) between the results obtained with fresh as contrasted with dried leaves, it was thought advisable to check the observations presented in Table I using fresh leaves from certain of the same plants involved in the tests

previously made. Opportunity was also taken to introduce one species each of the following families: Fagaceae, Leitneriaceae, Salicaceae, Juglandaceae, and Myricaceae. These other families of the "Amentiferae" were introduced in the hope that, should differences of reactivity within the group occur, some light might be shed on the vexing question of relationships between these families. A member of the Guttiferae was employed as a check on possible wider relationships. Again the observations (Table III) show absence of reactivity between the leaf tissue extracts.

To determine what reactive substances occur in the Betulaceae, in the Leitneriaceae, and in *Hypericum* of the Guttiferae, the concept of "test plant" of a "known" constitution was introduced. Fresh material of the species with the label-names indicated in Table IV was collected in the Arnold Arboretum and these were tested against each other. The results indicate a similar reactivity in *Betula populifolia, Diervilla florida,* and *Malus Arnoldiana,* since these show positive reactions only with *Iris chrysophoenicia, Nicotiana alata,* and *Lycopersicum esculentum,* and all contain an excess of calcium ion. This suggests the presence of but one principle of a reactive pair, probably "A" (3), since the *Nicotiana alata* and *Lycopersicum esculentum* have been shown in an earlier paper (3, p. 186, fig. IV) to contain an excess of the complement "B." The possibility of this being the "MN" reaction (l. c.) is eliminated because of the negative reactions with *Ligustrum obtusifolium* which is known from the earlier work to be "N+."

An interesting situation exists in the case of *Leitneria* where but one odd reaction occurred, namely with *Syringa velutina,* suggestive of a fifth reactive pair. Further investigation is needed here.

An analysis of the results presented in Tables I and III yields little of value in the light of these conclusions, beyond the fact that opposing members of reactive pairs are absent. One of these is probably "B" and another the counterpart of the unknown existing in *Leitneria.* This striking absence of reactivity within the Betulaceae might well be construed in confirmation of preexisting morphological evidence indicating close relationship between the members of the family. But in considering the "Amentiferae" with their similar negative reactions, it should be kept in mind that widely varying treatments of the relationships between the families composing this group exist. Our evidence, in the light of the hypothesis expressed in an earlier paper (2) and later accepted by Foster and Avery (5), i. e. that negative results indicate very close or very distant relationship, helps not at all in clarifying the inter-relationships of the "Amentiferae."

The genus *Hypericum* as represented here includes ten species of five different sections as recognized by R. Keller in Engler and Prantl (4). The species vary from the small herbaceous *H. boreale* (Britton) Bicknell, the larger herbaceous *H. perforatum* L. and *H. Ascyron* L., through the shrubby forms to the dwarf mountain shrub *H. Buckleyi* M. A. Curtis. The list includes one known hybrid *H. Arnoldianum* Rehder with both parents (*H. galioides* and *H. lobocarpum*) (7). All the species used were collected in the Arnold Arboretum and authenticated by the junior author.

The precipitation reactions in *Hypericum* show consistent negative results as may be seen in Table II. From this one may conclude that the genus is homogeneous regarding reactive substances or that they are absent. In Table III one species of *Hypericum* tested against the "Amentiferae" shows no positive reactions. This affords no clue to the situation. However, when tested against other representative families (Table IV) it will be seen that the species is highly reactive. Unfortunately a wide enough variety of "known" plants was not used in the present study to make a complete analysis possible. It will be seen (Table IV) that good positive reactions were obtained with *Iris chrysophoenicia* and with the solanaceous representatives, *Nicotiana alata* and *Lycopersicum esculentum*. Since these species did not react against each other but reacted consistently against other forms, one may conclude the reaction to be of similar quality and doubtless due to the presence of the same reactive substances. The reactions with the oleaceous members, *Syringa velutina* and *Ligustrum obtusifolium*, must be due to a second set of reactive principles, since the Oleaceae also reacted with the Solanaceae. In the same manner the positive reaction with *Lonicera Myrtillus* probably represents a third set of characters. The trace shown with *Philadelphus grandiflorus* is of doubtful character since this species did not react with any other plant, thus giving no indication of the reactive substances involved.

From the evidence presented one is justified in concluding the presence of three reactive principles in *Hypericum;* whether these fall into the categories of the reactive substances designated in an earlier paper (2) as "AB," "MN," and "XY" is not known. Although we cannot be sure that the same principles occur in all species of *Hypericum*, at least the species tested do not show the presence of any of the opposing characters. The known reaction, calcium-oxalate, is of no significance in indicating degree of relationship in this genus as is shown by the harmony of negative results obtained when tested against calcium

chloride. Thus it would seem that in the genus *Hypericum* the precipitation technique would be of little value in determining relationships within the group.

TABLE IV. PRECIPITATION REACTIONS IN REPRESENTATIVES OF ALL THE FAMILIES THUS FAR INTENSIVELY STUDIED WITH REGARD TO THE PRECIPITATION REACTION

Notations as in Table II. Experiments performed with fresh material. Extracts containing an excess of oxalate neutralized with calcium chloride and re-filtered before testing.

	Iris pseudacorus	I.chrysophoenicia	Nicotiana alata	Lycopersicum esculenta	Syringa volatina	Ligustrum obtusifolium	Malus Arnoldiana	Prunus serrulata	Philadelphus grandiflorus	Deutzia scabra plena	Diervilla florida	Lonicera myrtillus	Leitneria floridana	Betula populifolia	Hypericum lobocarpum	Calcium chloride .01M	Potass.oxalate .01M
Iris pseudacorus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
I.chrysophoenicia	0	0	0	0	++	+	+	0	0	0	+	++	0	++	+++	0	++
Nicotiana alata	0	0	0	0	+++	+	+	0	0	0	+	++	0	++	+++	0	++
Lycopersicum es.	0	0	0	0	++	+	+	0	0	0	+	+	0	+	++	+	0 (++)
Syringa volutina	0	++	+++	++	0	0	0	0	0	0	0	0	+	0	++	+++	0 (+++)
Ligustrum obtus.	0	+	+	+	0	0	0	0	0	0	0	0	0	0	++	0	+++
Malus Arnoldiana	0	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	+++
Prunus serrulata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+++
Philadelphus gr.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	t	0	0
Deutzia scabra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+++
Diervilla florida	0	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	+++
Lonicera myrtil.	0	++	++	+	0	0	0	0	0	0	0	0	0	0	+	+++	0 (++)
Leitneria florid.	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	+
Betula populifol.	0	++	++	+	0	0	0	0	0	0	0	0	0	0	0	0	++
Hypericum lobocarp.	0	+++	+++	+++	++	++	0	0	t	0	0	+	0	0	0	0	+++

We are now in a position to resolve more satisfactorily the question of the taxonomic significance of the precipitation reaction in plants. Within certain of the limited plant groups tested (families, subfamilies, and genera) there have been obtained numerous positive reactions.

These positive reactions show a marked correlation with the taxonomic positions of the plants involved (2, 3, 5). Within others of the limited groups tested (Caprifoliaceae, Oleaceae, "Amentiferae," Guttiferae) entirely negative results have been obtained. In at least three of these (Oleaceae, Betulaceae, and Guttiferae) the genera and species tested are usually considered rather closely related to one another and the negative findings may well be of significance indicative of this close relationship. In such groups as the Caprifoliaceae, which while rather heterogeneous still display uniformly negative reactions, one may only conclude that chemical differences demonstrated by the precipitation technique are not necessarily associated with gross morphological differences, since any category of characters used for classification may vary independently of any other, a conclusion consistent with the findings whenever any two techniques (e. g. morphology, cytology, anatomy, genetics, etc.) are used in a taxonomic study of a given group of plants.

As regards systematics on a larger scale, that is, phylogeny of the angiosperms taken as a whole, the precipitation technique is apparently not applicable in a differentiation of widely separated groups. This is evident when one considers Table IV which gives the results of inter-family tests of nine widely separated families of plants. Here no useful correlation of precipitation reaction with systematic position is to be seen. The reason for this is quite apparent when one considers the nature of the precipitation reaction.

The thousands of positive reactions which have been observed in fifteen families of plants do not represent thousands of specific reactions but rather represent a relatively small number of analyzable reactions, one of which has been chemically determined and three of which have to some extent been characterized. Two or three pairs of reactive substances suffice to account for all of the inter-family reactions of Table IV; four pairs of reactive substances accounted for all of the reactions of paper III of this series (Solanaceae et al.). The substances responsible for any given reaction seem to be rather widely distributed throughout the angiosperms. Thus (Table IV) the substance in the Solanaceae which reacts with oleaceous extracts is likewise present in *Iris*, and the oleaceous complement also in *Malus* and the Caprifoliaceae. This fact does not detract from the value of the precipitation reaction when confined to limited groups of plants (families, subfamilies, genera). Within such limited groups it has been found that morphologically similar species tend to carry similar complements of precipitating substances (2, 3, 5).

The value of the technique within families of the higher plants when used to determine similarity or difference as far as the three or more unknown and one known pairs of variables are concerned, is to be evaluated as in the case of any other category of evidence with a similar number of variables. If all four or more pairs of variables are present in a given family the technique is correspondingly significant. If the number of variables is less, the value of the technique is accordingly reduced, until we reach a condition such as exists in the Betulaceae and apparently the "Amentiferae" as a whole where the precipitation technique indicates no difference between the species tested.

It is obvious from an examination of the experimental data thus far obtained that the method in its present form with only a relatively few pairs of variables provides hardly enough combinations of characters to be of significant aid in determining the extent of relationship between widely separated families. Thus we must guard against a possible misinterpretation of the statement made earlier in this series of papers (2) and later confirmed by Foster and Avery (5) that absence of reaction indicates very close or very distant relationship, while a positive reaction indicates an intermediate degree of relationship. Clearly this concept cannot be applied to groups too diverse morphologically—its use must be restricted to plants closely related as determined by other means. Within such groups it should be kept in mind that the applicability of the method is determined by the number of distinct reactive pairs of substances. The greater the number of these the greater the number of categories into which the plants being investigated can be placed.

In pointing out this limitation of the applicability of the precipitation reaction in plant systematics let us compare it with the use of chromosome number in taxonomy. Within limited groups of plants chromosome number may aid in classifying species; the fact that the same chromosome number may be found in very distantly related plant groups does not detract from the use of chromosome number in plant classification. The same is true of the precipitation reaction; its applicability in closely related groups of plants is not to be belittled by the fact that the same reactive substance may occur in very distantly related plant families.

The work done up to the present on the precipitation reaction in plants marks only a groping beginning toward a phase of plant systematics which will doubtless develop more widely in years to come, namely, the use of chemical properties in the study of plant relation-

ships. In testing plants by the precipitation technique one is actually testing related groups of species against arbitrary and fortuitously selected "test extracts." Since there is apparently only a very limited number of types of precipitation reaction present in plants, one would actually gain in time and efficiency if he were to select a few "test species" containing various known reactive complements, and use these as standards against which to test all species of selected limited groups. That this "test species" concept has its limitations is shown by a comparison of the reactivity observed in Table IV with that reported by Foster and Avery and in previous papers of this series. The reaction indicated between *Iris chrysophoenicia* and *I. pseudacorus* is strictly negative according to our results but (+ +) according to Foster and Avery. Similar discrepancies exist in the behavior of *Solanum lycopersicum* and *Nicotiana alata* which in work earlier reported (3) were negative to oleaceous forms but were found (Table IV) in this series of experiments to be positive. It would seem that in order to define the constitution of an unknown plant according to the suggestion made in paper III of his series (p. 185), it is necessary in every new series of experiments to redetermine critically the constitution of "test extracts."

To go one step further, would it not be more satisfactory to submit the few reactions found to detailed biochemical analysis in order eventually to substitute for the "test species" simple chemical solutions of known composition, containing only the active ingredients of the "test extracts"? The senior writer has already done this with respect to one, namely the calcium oxalate reaction. A relatively small number of such solutions would enormously simplify the precipitation technique and would give results far more accurate and explainable than those thus far obtained. The inquiring botanist at this point must turn to the skilled biochemist for aid in resolving this problem. It is essential now that the precise nature of the precipitation reactions in plants be made the subject of investigations by someone adequately trained in analytical organic chemistry; the results of his research could not fail to be of value in advancing our knowledge of this phase of plant systematics.

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THE ARNOLD ARBORETUM DURING THE FISCAL YEAR ENDED JUNE 30, 1933

THE YEAR 1932-33 proved favorable to the growth of all collections in the arboretum and fruticetum. Late in February a heavy snow-fall caused considerable damage to Junipers, Spruces and small deciduous trees.

Four hundred forty-two plants were added to the collections; these were taken from the Arborway nursery.

Exchange of plants, cuttings, scions and seeds were continued and during the year 798 cuttings and scions, 2213 plants and 817 packets of seed were sent out. There were received from institutions in this country, Canada, Europe and Australia 1091 packets of seed, 1556 plants and 158 cuttings and scions.

Eleven hundred and thirty visitors registered at the Administration Building. Thirty-seven states in the Union were represented and also British Columbia, Nova Scotia, France, Switzerland, Holland, Germany, England, Ireland, China, Japan, Philippine Islands and Australia.—L. V. S.

Pathological Laboratory.—The Arboretum's research laboratory in Plant Pathology submits its report for 1932-3, making reference to certain new diseases, extension services and investigative activities.

One of the important functions of the pathologist's office is to advise of diseases that loom on the horizon, that is, of potentially devastating diseases which once established would entail heavy losses and constant warfare. An example of what the Arboretum has done in this connection is afforded by the warnings broadcast and the testing of effective control measures against the beech bark disease, a scourge that has within the last decade destroyed a high percentage of the beech trees in the adjacent Maritime Provinces and now is advancing into Maine. The predisposing factor of the disease, a bark-infesting insect similar to the woolly aphis, was discovered about four years ago by Mr. John Ehrlich of this laboratory to be sporadically distributed throughout metropolitan Boston. Subsequently, it was located elsewhere in eastern Massachusetts by other observers. Through the united efforts of the Arboretum and the Park Department it has been largely eradicated within the Boston area.

Two other diseases now demand notice, one an affection of Firs known as the "Gout disease," the other of Elms, the so-called "Dutch elm disease." The former was pointed out to me in Nova Scotia in 1929 by the Provincial Forester, Mr. Otto Schierbeck. Quoting from the Journal of the Arboretum, XI: 57. 1930 (Faull, J. H.: Notes on Forest Diseases in Nova Scotia)—"Standing out as perhaps most interesting of all is a widespread condition of Balsam Fir, involving practically all of the trees of entire stands, what I would designate for want of a better term as 'Gout.' The trees are stunted, the trunks taper rapidly and never reach normal height, the joints are very much swollen and the twigs in general are thickened and tend to be deflexed." To this might now be added that many trees are soon killed outright and others are grossly mis-shapen. At that time the etiology was not known, nor was it certain that a contagium was responsible. Through the researches of Mr. R. E. Balch of the Dominion Entomological Branch it has recently been determined that the cause is a bark insect of the woolly aphis type. In a recent eastern trip I observed that spread has been rapid and destruction extensive. It is found at present as far south as Brunswick, Maine. I am informed by Mr. H. B. Peirson, Entomologist for the State of Maine, that control is entirely practical in ornamentals by use of a contact insecticide. It is known to attack *Abies balsamea, A. arizonica, A. Fraseri, A. nobilis, A. glauca, A. sibirica* and certain other Firs.

Dutch elm disease is an even more serious malady because among the highly susceptible hosts is our much-beloved and widely-grown American Elm. From tests made in Holland on plants supplied by the Arboretum, Dr. C. Buisman has demonstrated that the American Elm is among the most vulnerable of the Elms. This disease is wide-spread throughout Europe and it has worked havoc there. Three years ago a few cases were discovered in Cleveland and one in Cincinnati; they were promptly extirpated. Report this year comes of an outbreak in New Jersey and southern New York which appears to be of much more serious proportions. The Arboretum is intensely interested in the problems of this and other elm diseases and will participate so far as its resources permit in efforts towards their solution.

The extension work of the laboratory, though more or less incidental to its main purpose, has continued to grow. A great many inquiries are received, and these are generally accompanied by specimens for diagnosis. To care for this phase of our work the necessity for adequate assistance in the laboratory, especially during the growing sea-

son, is a matter of importance. While much of the material that comes in to us can be readily disposed of, frequently more or less critical material is received that requires extended attention. Particularly has this been the case this year because of uneasiness among so many of our correspondents regarding the possibility of the presence of the Dutch elm disease, the certain diagnosis of which involves rather tedious laboratory technique. Consultation is welcomed, partly because of service that may be rendered, but also because unusual problems through this medium are from time to time disclosed.

The research undertakings of the laboratory have materially advanced during the year, yielding results of scientific interest and of economic application. A summary follows.

Professor J. H. Faull, continuing his studies on the rust fungi that attack conifers, has added to the literature a monograph on the hitherto little understood genus *Milesia* (Contributions from the Arnold Arboretum, No. II). These curious rusts, so far as has been determined, alternate between Firs and ferns. The monograph rectifies the tangled nomenclature, outlines the geographical distribution of species of *Milesia,* the world over, describes thirty-three species and two varieties, delineates thirteen new species and varieties, and records for the first time the life histories of three species of the nine whose life cycles are now more or less completely known.

Dr. K. S. Chester, on leave of absence in Europe on a Sheldon Fellowship for nine months of the year, has continued to publish on his work on precipitation reactions—on a biochemical analysis of these reactions and on the question of acquired reactions due to grafting. Most of his time has been devoted to an investigation of bacteriophage in relation to crown gall.

Dr. John Ehrlich has brought to a conclusion his immediate studies in America on the "beech bark disease" to which reference was made above. He now goes to Europe on a National Research Fellowship to study the problem at its original sources.

Miss L. M. Hunter has advanced her studies on the spermogonia of coniferous rusts and will continue her problem in Europe during 1933-4 supported by a traveling Fellowship.

Mr. I. H. Crowell has completed two years of intensive investigation on the Gymnosporangium rust diseases of *Juniperus* and the various Pomaceae other than Hawthorns. This work has been jointly supported by Dr. and Mrs. Henry Lyman and the Arboretum. Mr. J. D. MacLachlan is making parallel studies on *Juniperus* and the Hawthorns.

Mr. A. B. Hatch has devoted himself to a study of the mycorrhizae of Pines. He has made a notable contribution to the technique employed in the investigation of the biology of these remarkable structures and has published the first adequate account of the mycorrhizae of the White Pine. His investigations are conducted jointly under the auspices of the Harvard Forest and the Arboretum and have been made possible through the liberal generosity of an anonymous benefactor. — J. H. F.

Cytological Laboratory.—During the past year the cytological investigations have covered a wide field, including considerable work on the mechanism of chromosome pairing and division, as well as the cytological analysis of various families and genera from the standpoint of relationships and origins. An analysis of the mechanism of crossing over was presented before the International Genetics Congress and will appear in the Proceedings.

The behavior of segmental interchange chromosomes in *Tradescantia* has been studied and the results published in Genetics. A detailed study of chromosome pairing in *Larix* was completed by Mrs. Sax and published in Genetics. Mr. O'Mara completed his work in chromosome division in the pollen tube of *Lilium* and it has been published in the Botanical Gazette. Dr. Dermen's work on the origin and behavior of the nucleolus has been completed. A study of chromosome pairing in *Paeonia* has thrown some light on the mechanism of chromosome association.

Cytological work in connection with species and generic relationships has been completed for *Yucca* and *Agave*, *Ulmus*, *Cornus*, *Acer*, *Magnolia* and a considerable number of conifers. *Yucca* and *Agave* seem to be more closely related than the taxonomic grouping would indicate, but Dr. Dermen's work on *Cornus*, Mr. Foster's work on *Acer*, the chromosome numbers found in *Ulmus* and *Celtis*, the results obtained in the conifers by Mrs. Sax, and Dr. Whitaker's work on *Magnolia* and related genera all show a close correlation between chromosome characteristics and taxonomic grouping. Closely related families often have similar chromosomes. In the conifers most genera are different in chromosome morphology, but species within each genus have similar genoms.

The cytology of hybrids between Old and New World species have been studied in species hybrids of *Larix*, *Platanus* and *Campsis*. The Larix and Platanus hybrids are completely fertile and the Campsis

hybrid partially fertile, even though the original parental species in each case are morphologically distinct, and have been isolated from each other for very long periods of time.

Breeding work has been continued with Roses, Lilies and conifers. Crosses have also been tried between different genera of Pomoideae, some of which seem to be successful. In general the breeding work has been confined to crosses between closely related species. — K. S.

The Herbarium.—During the past fiscal year 16,377 mounted specimens have been added to the herbarium, bringing the total number up to 374,880 specimens. Of these accessions approximately 3800 came from the United States and Canada, 4000 from Central and South America inclusive of Mexico, 600 from Europe and western and central Asia, 1900 from eastern Asia, 1700 from southern Asia and Malaysia, 1200 from Africa, 2400 from Australasia and 400 were cultivated plants.

Among the more important collections received during the year the following may be mentioned: 3400 specimens collected by J. and M. S. Clemens on Mount Kinabalu, Borneo; 1270 specimens from Lingnan University collected chiefly in Hainan and southeastern China; 1100 specimens from H. H. Chung, Wuhan University, collected mostly in Fukien and Hupeh; 3350 South American specimens from the U. S. National Herbarium including a large number of Argentine plants collected by Venturi; also from the U. S. Herbarium 270 specimens collected by J. F. Rock in Yunnan and Burma; about 1000 numbers of Australian specimens collected by C. T. White, S. F. Kajewski and L. J. Brass; nearly 1000 numbers with about four sets of duplicates of plants from the Solomon Islands collected by L. J. Brass; 950 specimens of Kweichou plants from the University of Nanking; 900 specimens comprising 18 fascicles of the Herbarium Florae Rossicae from the Botanical Institute in Leningrad; 600 Brazilian plants collected by B. A. Krukoff; 575 plants from Colombia collected by A. E. Lawrance; over 550 plants from Trinidad collected by W. E. Broadway, 650 South African plants collected by F. A. Rogers; 1500 specimens chiefly from Indochina and Madagascar received from the Museum of Natural History at Paris; 195 specimens from Indochina collected by R. W. Squires.

To the fruit collection 370 specimens were added bringing the number up to 7815.

Additions of 451 specimens were made to the wood collection bringing the total up to 2816.

The collection of negatives of types and other important herbarium specimens consists now of 2524 negatives; 667 having been added, mostly types of Chinese species taken by the Curator in European herbaria.

Besides constantly using the herbarium in the determination of plants sent in for identification and also in the determination of large collections chiefly from eastern Asia and North America members of the staff have engaged in special work; Mr. E. J. Palmer is continuing his studies in the genus *Crataegus;* Dr. I. M. Johnston has published extensive notes on the Boraginaceae of the western United States; Mr. A. Rehder is continuing the revision of the ligneous plants described by H. Léveillé from Eastern Asia and Mrs. Susan D. McKelvey her studies in the genus *Yucca.* Among the visitors who have worked in the herbarium may be mentioned Dr. Shun Ching Lee of the National Normal University, Peiping, China, who has spent five months here in the preparation of a work on the forest trees of China, Dr. R. E. Woodson, Jr., of the Missouri Botanic Garden studied Apocynaceae and Mr. E. H. Walker, of the National Herbarium, Washington, Chinese Myrsinaceae; also Dr. L. H. Bailey of Ithaca, Dr. L. Diels, director of the Berlin Botanic Garden, Dr. S. F. Blake, Mr. W. W. Eggleston and Paul Russell of the Department of Agriculture, Washington, and Dr. A. Gundersen of the Brooklyn Botanic Garden consulted the herbarium.

For study outside the Arboretum herbarium 586 specimens were lent to 18 institutions and individuals in this country, Europe and China.

There have been distributed 14,536 specimens to 38 institutions in the United States, Canada, Europe, Asia, Africa and Australia.

Botanical exploration by members of the staff or by expeditions partly or wholly financed by the Arnold Arboretum has been carried on in both Americas, Eastern Asia and Australasia.

Dr. H. M. Raup and Mr. E. C. Abbe started in June 1932, as mentioned already in the last report, on a tour of botanical exploration of the Peace River region in the Canadian provinces of Alberta and British Columbia with financial aid from the National Research Council along with the support of the National Museum of Canada; they returned toward the end of September, having collected about 6400 specimens representing 1200 numbers. An account of the flora of this region has been prepared by Dr. Raup and will be published as No. VI of the Contributions from the Arnold Arboretum.

Professor J. G. Jack spent the time from the end of January to the beginning of April at the Atkins Institution of the Arnold Arboretum

at Soledad, Cuba, and collected in the neighborhood of Soledad and Cienfuegos about 600 numbers with many duplicates and with fruit and wood specimens.

Dr. I. M. Johnston left for England in October under a fellowship of the Guggenheim Foundation, chiefly to continue his work on the Boraginaceae and to study type specimens and other material of South American plants at Kew and the British Museum. In February he went to the Continent and worked first in Berlin and later in Paris.

Mr. Alfred Rehder spent the time from the end of June to the end of September in Europe for the purpose of examining and photographing type specimens of plants of China and the adjoining regions. He took about 600 photographs of types and critical specimens in the herbaria at Kew, the British Museum, Berlin, Florence, Geneva and Paris.

In China botanical expeditions of three institutions have had the financial support of the Arnold Arboretum. The southern province of Kwangsi, botanically as yet very little known, is being explored by an expedition from the University of Nanking under the direction of Dr. A. N. Steward. The Fan Memorial Institute of Biology at Peiping has, since spring 1932, an expedition collecting in the less well known parts of Yunnan; it also maintains an expedition in cooperation with the Academy of Science of Western China in Szechuan, which collected during 1932 about 2500 specimens and is in the field again this year under the direction of Dr. H. H. Hu. Lingnan University had under the direction of Dr. F. P. Metcalf during the second half of last year and the first half of this year an expedition in the field collecting in Hainan and Kwangtung which had up to the end of last year collected about 2125 numbers; another expedition started in January for Kwangsi and will remain there until July.

In the Solomon Islands Mr. L. J. Brass, who had already collected for the Arnold Arboretum in New Guinea from 1925 to 1926, continued the work of S. F. Kajewski and visited during the time from July 1932 to the beginning of January 1933 San Cristobal and Ysabel Island and also several smaller islands; he collected nearly 1000 numbers with approximately four sets of duplicates. In February he joined the Archbold Expedition to New Guinea.

In the summer of 1932 Dr. C. Regel, Director of the Botanic Garden at Kaunas, Lithuania, undertook a collecting tour to Asia Minor, partly financed by this Arboretum. Owing to the attitude of the Turkish authorities this trip was not very successful.

From April to the end of June 1932 Professor J. Bornmüller of Weimar made a successful collecting tour to Tripoli, Sicily and southern Italy with financial help from the Arnold Arboretum. — A. R.

The Library.—At the end of June 1933 the Library comprised 40,919 bound volumes, 10,085 pamphlets and 16,898 photographs, a gain of 271 volumes, 200 pamphlets and 112 photographs during the year. The increase, due to conditions, was smaller than in previous years but, nevertheless, presents an interesting breadth of inclusion, and more time could be given to analyzing important articles from periodicals and other works, thus enriching the material easily available.

Though few photographs have been added, the use of the collection has been extensive. Nearly 100 prints have been made and sold for nursery catalogues, post cards, publications, and collections institutional and private. Eleven photographs of Lilies were loaned to the Royal Horticultural Society of London for the Lily Conference. Eighty-eight prints of photographs taken in China by the late Dr. E. H. Wilson were made for Dr. Shun Ching Lee of Peiping, China, besides prints made for our own publications, and many photographs were used in lectures given by members of the staff. Eight colored slides reproduced from photographs in the collection have been added to the lantern slide collection and seven slides were made for the New York State College of Forestry. Slides used during the year number over 300.

Cards filed during the year include 737 in the card catalogue of books in the Library, 119 in the catalogue of photographs, 4,598 in the "Card-index of New Genera, Species and Varieties published by the Gray Herbarium," and 4,900 in the manuscript "Index of Illustrations and of New Genera, Species and Varieties of Ligneous Plants published since 1915," prepared at the Arboretum, bringing the total number of the latter to 97,639.

A very large amount of time has been spent in reading proof for the "Catalogue of the Library of the Arnold Arboretum of Harvard University, Volume iii, Serial Publications—Authors and Titles, Supplement, 1917-1933," which has now reached completion and is ready for distribution. It comprises 346 pages and approximately 17,300 entries arranged alphabetically, with numerous references. Nearly 900 slips have been filed for entries received too late for inclusion in the printed Catalogue and 300 slips for a supplement to the "Subject Catalogue," which is now in preparation.

Volumes bound number 113, as against 570 of last year, and nearly 100 smaller books and pamphlets were put into pamphlet binders.

Clipping files and scrap-books, with references in the card catalogue, bring together and preserve much interesting and valuable material which would otherwise be lost.

A large number of visitors have registered in the Library during the year. Dr. Shun Ching Lee of the National Normal University, Peiping, China, spent several months studying the forest trees of China. Professor L. H. Bailey and Miss Ethel Zoe Bailey, of Ithaca, New York, Mr. E. H. Walker, of the Smithsonian Institution, Dr. S. F. Blake and Mr. Paul Russell of the United States Department of Agriculture, Dr. R. E. Woodson, Jr., of the Missouri Botanical Garden, Dr. Conway Zirkle of the University of Pennsylvania, and Miss Elsie Jack of Hatzic, British Columbia, spent some time consulting the Library. Among other visitors were Mr. Frederick A. Delano to whom the Library owes its fine collection, previously described, of Chinese paintings of fruits and flowers, Dr. David Fairchild of Cocoanut Grove, Florida, and Mr. and Mrs. Arthur B. Spingarn of Amenia, New York.

The publications of the Arboretum, the "Journal of the Arnold Arboretum" and the "Arnold Arboretum Bulletin of Popular Information," were issued regularly; of the "Contributions from the Arnold Arboretum of Harvard University" numbers II-IV were published during the year. Of the approximately 400 periodicals that come to the Library from all parts of the world, nearly 250 were received in exchange for these publications.

More than 50 new periodicals have been received during the year, a large number in exchange for our publications and for herbarium specimens, some by gift and a number by purchase. They include:

AALSMEER—*Vereeniging tot oprichting en instandhouding van den proeftuin.* Jaarverslag. 1931. Aalsmeer. 1932.

AMERICAN civic annual. Vol. i-iv. Washington. 1929-32.

AMERICAN nurseryman. Vol. lvii, no. 2 → Rochester. 1933 →

AMERICAN orchid society bulletin. Vol. i, no. 1 → Washington. 1932 →

AMERICAN rose magazine. Vol. i, no. 1 → Harrisburg, Pa. 1933 →

BOLETIN de pro-cultura regional, S. C. L. Tom. i, n. 1-24, 28-30. Mazatlan. 1929-32.

BULLETIN of applied botany, of genetics and plant-breeding. Ser. A. Socialistic plant-industry. No. 1 → Leningrad. 1932 →

BULLETIN of applied botany, of genetics and plant-breeding. Ser. ii. Genetics, plant-breeding and cytology. No. 1 → Leningrad. 1932 →

FIELD museum news. Vol. ii, no. 10, 12; iii, 1-3, 5-11; iv, 1 → Chicago. 1931 →

FLORALIA. Vol. liv, no. 13 → Haarlem. 1933 →

FOREST log. Vol. i, no. 10-12; ii, 1; iii, 2 → Salem, Oregon. 1931 →

FORTSCHRITTE der botanik. Bd. i, 1931. Berlin. 1932.

GARDEN lover. Vol. ix, no. 1 → Melbourne; Sydney. 1931 →

HARVARD UNIVERSITY—*Farlow herbarium of cryptogamic botany.* Contributions. Vol. [i] → Boston, etc. 1883 → (Incomplete)

HEMLOCK ARBORETUM at "Far Country." Bulletin. No. 1 → Germantown, Pa. 1932 →

IDAHO forester. Vol. xiii → Moscow, Idaho. 1931 →

KAKTEEN-KUNDE. Heft 1 → Berlin. 1933 →

Forms new series of "Monatsschrift der Deutschen kakteen-gesellschaft."

KAUNAS—*Universitas Vytauti Magni—Hortus botanicus.* Scripta. Tom. i → Kaunas. 1931 →

KIVU—*Laboratoire des produits végétaux et de l'herbier du Service forestier.* Communications. No. 1 → Bruxelles. 1930 →

LENINGRAD—*Akademiya nauk—Byuro po evgenike.* Izvestiya. No. 1-9. Petrograd. 1922-32.

MAKILING echo. Vol. xi, no. 2, 4. Laguna. 1932.

MINNESOTA studies in plant science. No. 1 → Minneapolis. 1932 →

NATIONAL nut news. Vol. xv, no. 1 → Chicago. 1932 →

REVISTA española de biologia. Tomo i, cuaderno 1 → Madrid. 1932 →

REVUE agrologique et botanique du Kivu. No. 1 → Bruxelles. 1932 →

SAGHALIEN CENTRAL EXPERIMENT STATION. Reports. Ser. 2. Forestry. No. 1 → Konuma. 1932 →

SOCIEDAD ESPAÑOLA DE HISTORIA NATURAL. Reseñas cientificas. Tom. vi, num. 1 → Madrid. 1931 →

TAIHOKU—*Imperial university—Faculty of science and agriculture.* Annual report of the Taihoku botanic garden. No. 1 → Taihoku. 1931 →

TOKYO bunrika daigaku. Science reports. Section B. Vol. i, no. 4-10. Tokyo. 1932.

WARSAW—*Université—Institut de botanique systématique et de phytogéographie.* Publications. No. 2, 6, 7, 10, 12, 13, 15-17, 19, 20, 22-25, 27 → Cracovie, *etc.* 1927 →

On November 8, 1932, fifty-five periodical titles were sent for publication in the "Union List of Serials."

Among other important accessions are:

AMES, Mrs. Blanche Ames. Genera of the Gymnospermae, [Monocotyledoneae, Archichlamydeae and Metachlamydeae or Sympetalae], with their more important economic species arranged after Engler & Gilg. N. P. [1916-17.] 4 photographs.

Reproductions of charts made by Mrs. Ames for the use of classes in economic botany at Harvard University, showing relationships of economic plants given in the form of family trees.

[IWASAKI, Tsounemassa. Phonzo zoufou. Index. 1, 2. Yedo. 1916?]

LANCKMAN, C. Catalogue d'une belle et riche collection de rosiers à fleurs doubles; ainsi que toutes d'arbres à fruit, plantes de bruyère, d'orangerie, et un grand nombre d'arbustes et plantes de pleine terre. Gand. 1817.

LOWE, Edward Joseph. Beautiful leaved plants. By E. J. Lowe assisted by W. Howard. London. 1864. 60 colored plates.

CONGRÈS INTERNATIONAL DE BOTANIQUE ET D'HORTICULTURE, *Paris*, 1878. Comptes rendus. Paris. 1880.

SCHWIMMER, Johann Michael. Ex physica secretiori curiositates, non minus utiles, quam jucundae. Jenae. 1672.

"A collection of very remarkable discourses on various subjects of natural history. Most remarkable is chapter vii, "Conjugium et sexus duplex vegetabilium," which proves Schwimmer to have been a very early forerunner of Linné, whose "Sponsalia plantarum" did not appear till 1746. The earliest dissertation, "De sexu plantarum," mentioned by the bibliographers is that by Camerarius, 1694, i. e., twenty-two years later than this book."

[ELLIOTT, Stephen. Botanical manuscript. 1810-14.]

This appears to be an early draft of his "Sketch of the botany of South Carolina and Georgia," which was much altered and enlarged before printing.

[———— Letters to, and other manuscripts by, Stephen Elliott. 1790-1829.]

These manuscripts together with the preceding, given to Professor C. S. Sargent many years ago, have but recently come to light and they prove to be of considerable interest. The letters comprise thirty from Henry Muhlenberg, 1808-1815, written in a very fine hand on foolscap paper, one from Zaccheus Collins, 1815, informing Elliott of Muhlenberg's death. Four are from William Darlington, 1827, twenty-four from Dr. James MacBride, one from George Arnott Walker Arnott, 1828, one from William Prince, 1828. Twenty-seven letters are from S. Boykin, F. Boie, J. Vaughan, Wm. Swainson,

Wm. Thouin, Thomas Say, Dr. Lewis Schweinitz, John Brace, and others. An interesting short letter is from John Abbot, 1817, regarding some of his drawings of insects. Several thousands of Abbot's drawings exist in Europe. The British Museum has seventeen stout quarto volumes of them all bought from Francillon, a silversmith in London, and carry Francillon's name, book stamp and printed title-pages, dated 1792-1804. There are also volumes of them in the museums of Oxford, Paris, Zurich and elsewhere.

"Lists of books for the Charleston Library demanded by Stephen Elliott, Esq.," invoices, and letters signed by F. A. Michaux, lists of plants, expense accounts, bill of lading signed by Baudry, captain of the French brig Danurge, 1829, and twenty-one "Proposals for publishing by subscription a work on the botany of South Carolina and Georgia, by Stephen Elliott," with signatures of subscribers, complete the collection of miscellaneous manuscripts.

In addition to the Elliott letters the Library possesses several thousand letters from various writers addressed to different members of the staff of the Arnold Arboretum. These comprise letters from many eminent botanists, from collectors during their expeditions for the Arboretum, and other important letters on botanical matters. To arrange these satisfactorily will be part of the undertaking for the coming year.

The collection of nursery catalogues is becoming an important department and an effort has been made to obtain the latest catalogues of as many firms as possible.

A large number of books were sent out as inter-library loans to Gray Herbarium, Bussey Institution, Harvard Medical School, Harvard Biological Laboratory, Harvard Forest; Harvard College, United Fruit Company, Michigan State College, University of Iowa, West Virginia University, Canada Department of Mines, Tufts College, Marine Biological Laboratory, Yale Forestry School, New York State College of Agriculture, Dartmouth College, Smithsonian Institute, Massachusetts Institute of Technology, McGill University, University of Pennsylvania, American Museum of Natural History, Antioch College, University of New Hampshire, Massachusetts State College, Texas Agricultural Experiment Station, Forest Products Laboratory, Madison, Wisconsin, University of Toronto, and Wellesley College.

In addition to loans, photostats or typewritten copies of references have frequently been made when books could not be loaned.

Fifteen books were borrowed for members of the staff from the libraries of the United States Department of Agriculture, Cornell University, Ohio State University, Harvard College, Pennsylvania Academy of Natural Science, Massachusetts Horticultural Society, and Gray Herbarium. — E. M. T.

Bibliography of the published writings of the staff and students
July 1, 1932–June 30, 1933

ALLEN, Caroline Kathryn.

A monograph of the American species of the genus Halenia. (In *Annals of the Missouri botanical garden*, 1933, xx, 119–222.)

AMES, Oakes.

The Arnold arboretum; (report 1931–32). (In *Journal of the Arnold arboretum*, 1932, xiii, 447–448.)

The Cattleyeae. (In *American orchid society bulletin*, 1932, i, 37–39.)

An extension of range for Epidendrum rhynchophorum. (In *Harvard university, Botanical museum leaflets*, 1932, no. 3, pp. 1–4.)

Insect visitors to Cypripedium parviflorum. (In *American orchid society bulletin*, 1932, i, 91.)

John Lindley (1799–1865). (In *American orchid society bulletin*, 1932, i, 34–36.)

New or noteworthy Philippine orchids. iii. (In *Philippine journal of science*, 1932, xlix, 483–504.)

A new variety of Laelia Digbyana. (In *American orchid society bulletin*, 1932, i, 59.)

An addition to the genus Epidendrum. (In *Harvard university, Botanical museum leaflets*, 1933, no. 7, pp. 1–4.)

An addition to the genus Hexadesmia. (In *Harvard university, Botanical museum leaflets*, 1933, no. 6, pp. 1–3.)

Additional notes on the orchids of the New Hebrides and Santa Cruz Islands. (In *Journal of the Arnold arboretum*, 1933, xiv, 101–112.)

The Honduran species of Lepanthes. (In *Harvard university, Botanical museum leaflets*, 1933, no. 4, pp. 3–8.)

A new Bletia from Spanish Honduras. (In *Harvard university, Botanical museum leaflets*, 1933, no. 6, pp. 5–7.)

A new Epidendrum from Honduras. (In *Harvard university, Botanical museum leaflets*, 1933, no. 8, pp. 1–3.)

A new Octomeria from Spanish Honduras. (In *Harvard university, Botanical museum leaflets*, 1933, no. 4, pp. 1–3.)

A new Oncidium from Honduras. (In *Harvard university, Botanical museum leaflets*, 1933, no. 5, pp. 1–5.)

The Reichenbachian herbarium. (In *American orchid society bulletin*, 1933, i, 98–99.)

ANDERSON, Edgar.

Botanizing from an airplane. By Edgar Anderson and Oliver Ames. (In *Arnold arboretum bulletin of popular information*, 1932, vi, 37–44.)

Growing oranges in Boston. (In *Arnold arboretum bulletin of popular information*, 1932, vi, 45–47.)

The propagation of woody plants. By Edgar Anderson and W. H. Judd. (In *Arnold arboretum bulletin of popular information*, 1932, vi, 53–56.)

Basswood bark and its use by the Indians. (In *Arnold arboretum bulletin of popular information*, 1933, i, 33–37.)

Color variation in a Missouri colony of Hepatica acutiloba. (In *Rhodora*, 1933, xxxv, 66–67.)

Distribution of Iris versicolor in relation to the post-glacial Great Lakes. (In *Rhodora*, 1933, xxxv, 154–160.)
Jabberwocky [a discussion of botanical names]. (In *Arnold arboretum bulletin of popular information*, 1933, i, 41–44.)
Leatherwood. (In *Arnold arboretum bulletin of popular information*, 1933, i, 25–27.)
Plants of current interest. (In *Arnold arboretum bulletin of popular information*, 1933, i, 28–32, 37, 51-52.)
The River Birch (Betula nigra). By Edgar Anderson and E. J. Palmer. (In *Arnold arboretum bulletin of popular information*, 1933, i, 6–8.)
Segmental interchange in chromosomes of Tradescantia. By Karl Sax and Edgar Anderson. (In *Genetics*, 1933, xviii, 53–67.)
Shrubs attractive in late winter and early spring. (In *Arnold arboretum bulletin of popular information*, 1933, i, 17–20.)
A simple device for exhibiting flowering shrubs. (In *Arnold arboretum bulletin of popular information*, 1933, i, 50–51.)
Trumpet-creepers. (In *Arnold arboretum bulletin of popular information*, 1933, i, 1–5).
Vicinism in Aquilegia vulgaris. (In *American naturalist*, 1933, lxvii, 190–192.)

Bailey, Irving Widmer.
The cambium and its derivative tissues. vii. Problems of identifying the wood of mesozoic Coniferae. (In *Annals of botany*, 1933, xlvii, 145–157.)
Investigation on cambium and its derivative tissues. (In *Yearbook of the Carnegie institution of Washington*, 1932, xxxi, 191–192.)
Preliminary notes on cribriform and vestured pits. (In *Tropical woods*, 1932, no. 31, pp. 46–48.)

Chester, Kenneth Starr.
Grafting. (In *Arnold arboretum bulletin of popular information*, 1932, vi, 48–52.)
The problem of acquired physiological immunity in plants. i. (In *Quarterly revue of biology*, 1933, viii, 129–154.)
Studies on the precipitin reaction in plants. iii. A biochemical analysis of the normal precipitin reaction. By K. S. Chester and T. W. Whitaker. (In *Journal of the Arnold arboretum*, 1933, xiv, 118–197.)
Studies on the precipitin reaction in plants. iv. The question of acquired reactions due to grafting. By T. W. Whitaker and K. S. Chester. (In *American journal of botany*, 1933, xx, 297–308.)

Currie, Mary E.
Parasitism of myxomycete plasmodia on fungous mycelia. By F. L. Howard and Mary E. Currie. (In *Journal of the Arnold arboretum*, 1932, xiii, 438–447.)

Dermen, Haig.
Cytological studies of Cornus. (In *Journal of the Arnold arboretum*, 1932, xiii, 410–416.)

Ehrlich, John.
The beech bark disease in Nova Scotia. (In *Province of Nova Scotia, Report of the Department of lands and forests*, 1932, pp. 39–45.)
Nectria coccinea on beech. (In *Phytopathology*, 1933, xxiii, 10.)

FAULL, Joseph Horace.

Arnold arboretum Pathological laboratory; (report 1931–32). (In *Journal of the Arnold arboretum,* 1932, xiii, 448–450.)

Taxonomy and geographical distribution of the genus Milesia. (In *Contributions from the Arnold arboretum of Harvard university,* 1932, ii.)

HATCH, Alden Bruce.

Mycorrhizal and other features of the root systems of Pinus. By A. B. Hatch and K. D. Doak. (In *Journal of the Arnold arboretum,* 1933, xiv, 85–99.)

Pure-culture technique for quantitative studies of plant growth in association with microörganisms. (In *Phytopathology,* 1933, xxiii, 14.)

True mycorrhizal fungus in contrast to Mycelium radicis atrovirens. (In *Phytopathology,* 1933, xxiii, 14.)

HOWARD, Frank L.

Myxomycete plasmodia on fungous mycelia. By F. L. Howard and Mary E. Currie. (In *Journal of the Arnold arboretum,* 1932, xiii, 438–447.)

JOHNSTON, Ivan Murray.

Studies in the Boraginaceae. ix. (In *Contributions from the Arnold arboretum of Harvard university,* 1932, iii.)

JUDD, William Henry.

The Great Tree of Tule. (In *Gardeners' chronicle,* 1932, xcii, 422.)

Hardy coniferous evergreens for New England. (In *Gardeners' chronicle of America,* 1932, xxxvi, 307–308, 314.)

The propagation of woody plants. By Edgar Anderson and W. H. Judd. (In *Arnold arboretum bulletin of popular information,* 1932, vi, 53–56.)

Raising the Dove Tree from seed. (In *Horticulture,* 1932, x, 386.)

KELLEY, Katharine Eleanor.

Bibliography of the published writings of the staff and students July 1, 1931—June 30, 1932. (In *Journal of the Arnold arboretum,* 1932, xiii, 458–461.)

KING, Edward.

Chromosome behaviour in a triploid Tradescantia. (In *Journal of heredity,* 1933, xxiv, 253–256.)

KOBUSKI, Clarence Emmeren.

Enumeration of the ligneous plants collected by J. F. Rock on the Arnold arboretum expedition to northwestern China and northeastern Tibet (additions and continuation). By Alfred Rehder and C. E. Kobuski. (In *Journal of the Arnold arboretum,* 1932, xiii, 385–409.)

An enumeration of the herbaceous plants collected by J. F. Rock for the Arnold arboretum. By Alfred Rehder and C. E. Kobuski. (In *Journal of the Arnold arboretum,* 1933, xiv, 1–52.)

MCKELVEY, Mrs. Susan Delano.

A white pine blister rust demonstration. (In *Horticulture,* 1932, x, 331.)

Taxonomic and cytological relationships of Yucca and Agave. By Susan D. McKelvey and Karl Sax. (In *Journal of the Arnold arboretum,* 1933, xiv, 76–81.)

O'MARA, Joseph.

Division of the generative nucleus in the pollen tube of Lilium. (In *Botanical gazette,* 1933, xciv, 567–579.)

PALMER, Ernest Jesse.
Crataegus arnoldiana, Arnold Thorn. (In *Addisonia,* 1932, xvii, 33–34.)
The Crataegus problem. (In *Journal of the Arnold arboretum,* 1932, xiii, 342–362.)
The ferns and fern allies of Missouri. By E. J. Palmer and J. A. Steyermark. (In *American fern journal,* 1932, xxii, 105–122.)
Leaves from a collector's note book. (In *Journal of the Arnold arboretum,* 1932, xiii, 417–437.)
The River Birch (Betula nigra). By Edgar Anderson and E. J. Palmer. (In *Arnold arboretum bulletin of popular information,* 1933, i, 6–8.)
Some hardy southern trees and shrubs. (In *Arnold arboretum bulletin of popular information,* 1933, i, 45–49.)
Trees used by the pioneers. (In *Arnold arboretum bulletin of popular information,* 1933, i, 38–39.)
RAUP, Hugh Miller.
Lichens. (In *Arnold arboretum bulletin of popular information,* 1933, i, [9–16].)
Range conditions in the Wood Buffalo Park of western Canada with notes on the history of the wood bison. (In *American committee for international wild life protection.* Special publication, 1933, i, no. 2.)
REHDER, Alfred.
Arnold arboretum Herbarium; (report 1931–32). (In *Journal of the Arnold arboretum,* 1932, xiii, 451–453.)
Enumeration of the ligneous plants collected by J. F. Rock on the Arnold arboretum expedition to northwestern China and northeastern Tibet (additions and continuation). By Alfred Rehder and C. E. Kobuski. (In *Journal of the Arnold arboretum,* 1932, xiii, 385–409.)
New species, varieties and combinations from the herbarium and the collections of the Arnold arboretum. (In *Journal of the Arnold arboretum,* 1932, xiii, 337–341.)
Notes on the ligneous plants described by H. Léveillé from eastern Asia. (In *Journal of the Arnold arboretum,* 1932, xiii, 299–332.)
A second species of Gossypiospermum. (In *Tropical woods,* 1932, no. 32, pp. 6–7.)
Uniformity in plant names. (In *Horticulture,* 1932, x, 460.)
An enumeration of the herbaceous plants collected by J. F. Rock for the Arnold arboretum. By Alfred Rehder and C. E. Kobuski. (In *Journal of the Arnold arboretum,* 1933, xiv, 1–52.)
Supplement to C. T. White, Ligneous plants collected in the territory of Papua (British New Guinea) in 1925–26 by L. J. Brass. (In *Journal of the Arnold arboretum,* 1933, xiv, 62–67.)
SAX, Hally Jolivette.
Chromosome pairing in Larix species. (In *Journal of the Arnold arboretum,* 1932, xiii, 368–374.)
Chiasma formation in Larix and Tsuga. (In *Genetics,* 1933, xviii, 121–128.)
SAX, Karl.
Arnold arboretum Cytological laboratory (report 1931–32). (In *Journal of the Arnold arboretum,* 1932, xiii, 450–451.)

Chromosome relationships in Pomoideae. (In *Journal of the Arnold arboretum*, 1932, xiii, 363–367.)

Meiosis and chiasma formation in Paeonia suffruticosa. (In *Journal of the Arnold arboretum*, 1932, xiii, 375–384.)

Recent advances in cytology. By C. D. Darlington. (Review.) (In *Collecting net*, 1932, vii, 201–203.)

Chromosome numbers in Ulmus and related genera. (In *Journal of the Arnold arboretum*, 1933, xiv, 82–84.)

Segmental interchange in chromosomes of Tradescantia. By Karl Sax and Edgar Anderson. (In *Genetics*, 1933, xviii, 53–67.)

Taxonomic and cytological relationships of Yucca and Agave. By Susan D. McKelvey and Karl Sax. (In *Journal of the Arnold arboretum*, 1933, xiv, 76–81.)

TUCKER, Ethelyn Maria.

Arnold arboretum Library; (report 1931–32). (In *Journal of the Arnold arboretum*, 1932, xiii, 453–458.)

WHITAKER, Thomas Wallace.

Fertile gourd-pumpkin hybrids. (In *Journal of heredity*, 1932, xxiii, 427–430.)

The chromosome complement of Cyphomandra betacea. (In *Journal of the Arnold arboretum*, 1933, xiv, 113–117.)

Cytological and phylogenetic studies in the Cucurbitaceae. (In *Botanical gazette*, 1933, xciv, 780–790.)

Studies on the precipitin reaction in plants. iii. A biochemical analysis of the normal precipitin reaction. By K. S. Chester and T. W. Whitaker. (In *Journal of the Arnold arboretum*, 1933, xiv, 118–197.)

Studies on the precipitin reaction in plants. iv. The question of acquired reactions due to grafting. By T. W. Whitaker and K. S. Chester. (In *American journal of botany*, 1933, xx, 297–308.)

The above articles cover a total of about 882 pages.

K. E. K.

Staff of the Arnold Arboretum, 1933–34

Oakes Ames, A.M., Arnold Professor of Botany, Supervisor.

John George Jack, Assistant Professor of Dendrology.

Alfred Rehder, A.M., Curator of the Herbarium.

Joseph H. Faull, Ph.D., Professor of Forest Pathology.

Irving Widmer Bailey, Sc.D., Professor of Plant Anatomy.

Karl Sax, Sc.D., Associate Professor of Cytology.

Edgar Anderson, Sc.D., Arborist.

Ivan Murray Johnston, Ph.D., Research Associate.

Clarence E. Kobuski, Ph.D., Assistant Curator, Herbarium.

Ernest J. Palmer, Collector and Research Assistant.

Caroline K. Allen, Ph.D., Assistant in the Herbarium.

Hugh M. Raup, Ph.D., Research Assistant.

Haig Dermen, Ph.D., Research Assistant.

Ivan H. Crowell, A.M., Assistant in Phytopathology.

Ethelyn Maria Tucker, Librarian.

Susan Delano McKelvey, Research Assistant.

Ethel Antoinette Anderson, Business Secretary.

Katharine Eleanor Kelley, Assistant in the Library.

Louis Victor Schmitt, Superintendent.

William Henry Judd, Propagator.

ERRATA AND ADDENDA

Page 14, line 9 *for* **brevicornu** *read* **pubescens**
" " line 11 *for* **pubescens** *read* **brevicornu**
" " line 22 **Bocconia** spec., change to **Macleaya microcarpa** (Maxim.) Fedde
" 15, line 7 **Meconopsis** spec., no. 13077 = **M. racemosa** Maxim? forma
" 15, line 8 **Meconopsis** spec., no. 14430 = **M. punicea** Maxim.
" 28, after line 6 from below insert:

Gentiana Piasezkii Maxim.

Southwestern Kansu: Tao River basin, no. 13186

" 31, line 14 from below refer no. 14181 to **Stachys Sieboldii** Miq. (fide Hand.-Mazz.)
" 35, line 4 **Pedicularis** spec., no. 12255 = **P. semitorta** Maxim.
" 38, line 21 strike out (*L. conglobatum* × *leontopodioides*)
" 59, lines 9 and 11 from below *for* 60 m. *read* 200 m.
" 65, line 14 from below *for* **Rhaphidophera** *read* **Raphidophora**
" 70, line 10 *for* 3/4 *read* 1/3
" 70, line 5 from below *for* 1/4 *read* 2/3
" 80, line 23 at end of line *for* chromosomes *read* chromosome
" 109, line 14 *for* LXIV *read* LXXIV
" 121, line 4 from below *for* 000 and 000 *read* 147 and 174
" 123, line 8 *for* 000 *read* 151
" 207, line 14 from below add **Synon. nov.**
" 227, line 13 from below *for* **Chickrassia** *read* **Chukrasia**
" 227, after line 1 from below insert:

Cipadessa cinerascens (Pellegr.) Handel-Mazzetti in Symb. Sin. VII. 632 (1933).

" 228, lines 5, 6, 11 and 17 from below and page 229, line 2 for *Cavalierella* read *Cavaleriella*
" 231, line 7 *for* 2659 *read* 3500
" 236, line 3 after *Bodinieri* insert Léveillé

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