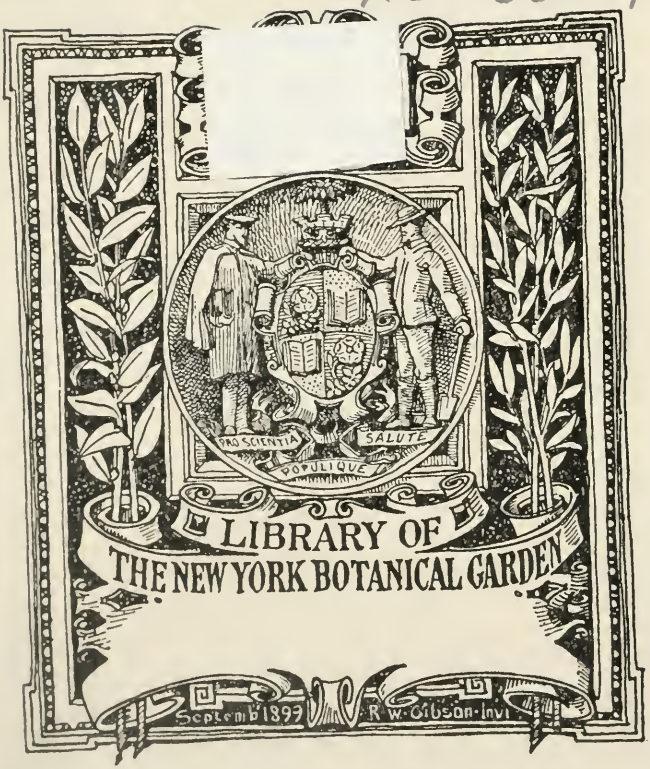
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PHYTOGEOGRAPHY OF THE EASTERN MOUNTAIN-FRONT IN COLORADO

I. PHYSICAL GEOGRAPHY AND DISTRIBUTION OF VEGETATION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 251

(WITH SEVENTEEN FIGURES)

ARTHUR G. VESTAL

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Introduction

The plant geography of a region is the effect of the working of present and former environmental influences upon the floras and vegetation-complexes which exist and have existed within the region and in the regions adjoining. The region of present study, lying as it does in the transition belt between two great geographic divisions of North America, the Great Plains, or western part of the prairie region, and the Rocky Mountains, has some of the characters of both; others of its physical and vegetational features are transitional, intermediate; and it has certain peculiarities, differing thus from the regions on either side. Since climatic variation, differences of soil and of topography, and multiformity of vegetation-types are considerable, the plant-covering of the area is a complex of many diverse types. Descriptive accounts of the plant associations of plains and foothills have already been published (17, 18), so that the present article may deal more particularly with geographic description and geographic relations.

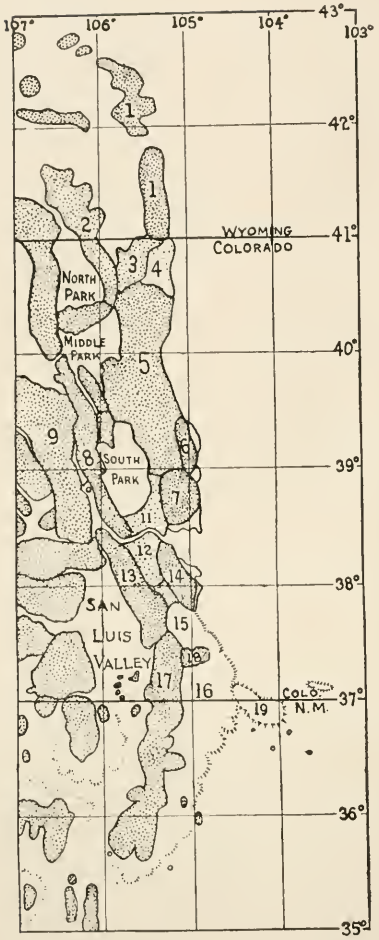


FIG. 1.—Map of southern Rocky Mountains, except westernmost ranges; mountain areas shaded; names of areas indicated by numbers are: 1, Laramie Mountains; 2, Medicine Bow Range; 3, low mountain area connecting Laramie and Front ranges; 4, foothills of Poudre River area; 5, Front Range; 6, Rampart Range; 7, Pike's Peak highland; 8, Park Range; 9, Saguache Range; 10, Upper Arkansas Valley (between 9 and 8); 11, low mountains; 12, Wet Mountain Valley; 13, Sangre de Cristo Range; 14, Wet and Greenhorn mountains; 15, Huerfano Park; 16, southern sedimentary plateau; 17, Culebra Range; 18, Spanish Peaks highland; 19, Raton mesas.

While in general the plains and mountains contrast rather sharply at their junction, this is not always true; the mountain-front is a transition zone in places a number of miles broad rather than a line. It is not determined alone by altitude, by topography, by character of the bedrock, or by climate; it is the resultant of all of these. For the sake of clearness the foothills may be described as the drier and less elevated (about 5800–8000 ft.) part of the mountain plateau, with vegetation composed of grassland, scattered rock pines, and a few other trees (*foothill zone*, RAMALEY 8). Except in the southern "sedimentary plateau" (fig. 1), perhaps rather to be considered part of the mountain-front area, the foothills may be said to comprise the granitic hills of the mountain-mass proper; while to the mountain-front zone may be assigned the upturned sedimentary hogbacks and longitudinal valleys, sedimentary outcrops, buttes and broken plateaus, and the mesas and upper parts of the débris-covered slope to the plains. The vegetation is of the greatest variety. The plains proper may be said to commence where the mixed soil and

vegetation of the detrital outwash from the hills is succeeded by the fine soil and mostly short-grass vegetation of the shale beds covering most of the Great Plains surface.

Plan of presentation.—The writer has been much influenced by the work of DAVIS (1) on the geography of the Colorado Front Range, a regional presentation and particularly relevant in this study, since the area considered is so nearly the same. DAVIS' systematic treatment avoids repeating descriptions of frequently encountered land-forms by recognizing their common features and giving each a brief characterization and a name, thus identifying them when mentioned later. Minor differences of detail are not considered in the condensed treatment thereby made possible. In a regional study, in which numerous elements form an intricate complex, this omission of detail is essential. As the physical geographer refers land-forms to types (mental counterparts of physical realities), so in a regional study of plant geography one may refer forms of vegetation to types which are the same over considerable areas. This is a common practice in ecological classification, but many studies of limited areas of vegetation have characterized the plant communities without regard to geographic orientation. If possible, local representations or variants of widespread associations should be recognized as such. The characterization of the relatively few widespread and important vegetation-types makes it possible to systematize plant geography. This systematic treatment emphasizes the common features, the resemblances of similar plant communities, but the differences, when worthy of note, can always be stated in addition. The section of this study which is here published is the systematic part, which establishes the types of topography, soil, climate, and vegetation as developed in the region or in parts of it. It will be followed by a regional section, which describes the physical and vegetational features "in their actual spatial relations," to use the words of DAVIS, and by parts dealing with general geographic and developmental relations of the vegetation.

Physical features

The area studied is the eastern front of the Rocky Mountains in Colorado, of which the most characteristic part is the Front

Range. This has been studied by many geographers, more recently by DAVIS. The Front Range has been described by him as a submaturely dissected upland of crystalline rocks, elevated above the plains to the east by a long north-south monoclinal fold. The tops of most of the hills form the remains of a peneplaned surface, the result of the erosion following the uplift, with complete removal of the sedimentary layers from the raised area on the west. A few

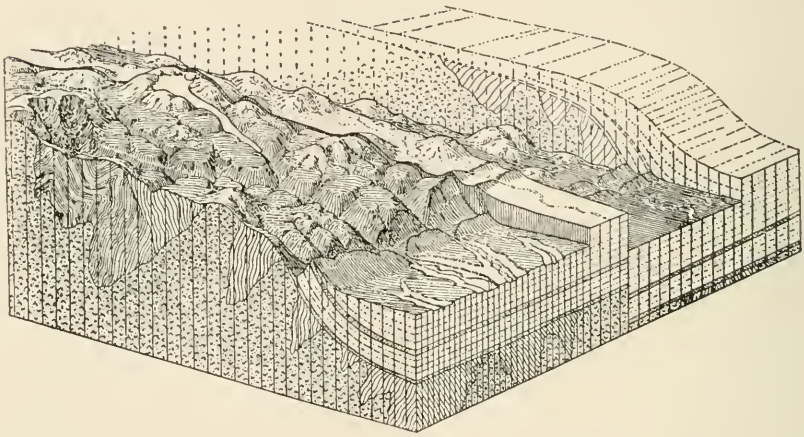


FIG. 2.—DAVIS' block diagram of Front Range (reproduced with author's permission from 1): at right is condition following first uplift with monoclinal fold; next part shows peneplaned upland with monadnocks and cuestas (hogbacks); third shows entire region after second uplift; last block on left shows present condition, with glacier-carved range-crest, gently sloping, dissected, crystalline upland, of which lower and eastern part forms foothills, and mountain-front, with sloping crags, cuestas, and longitudinal valleys; outside may be seen débris-covered terraces and broad valleys of streams running out into plains.

monadnocks surmount the general level. The present eastward inclination of the old peneplain and its dissected character in the crystalline area, and the removal of sedimentary strata of the plains to a depth far below the foothills, are the effects of a second uplift, an uparching of the whole region, and of the subsequent cycle of erosion. Near the base of the original fold the sedimentary strata are sharply upturned against the outer granitic slopes, the ends of the resistant strata forming ridges and sloping crags (fig. 2).

The outer slope to the plains has been described by JOHNSON (5) as a débris-apron or composite of alluvial fans, of which the profile is that of a stream-grade, rapidly flattening into the very slight and uniform incline of the Great Plains. The graded surface is covered by unassorted rock-waste from the hills, which thins out and becomes finer in texture toward the east; it is absent from most of the surface of the plains, which is of fine grained residual soil. This grade is that of the High Plains;[1] the streams have very generally cut below it, especially near the mountains. The Platte and Arkansas rivers, the trunk-streams, have cut very broad valleys in the soft shales of the plains. The north-south valleys of their tributaries which parallel the mountain-front are bordered on the east by escarpments of considerable height and are notable geographic features. This recent downcutting, where working in soft shales just outside the foothills, leaves many terraces, remnants of the older and higher stream-grade levels; their covering of rock-waste preserves their flat tops. They are generally known as "mesas"; although not true mesas, the term is convenient.[2] Where the upper sedimentary beds consist of sandstone or limestone, extensive plateau areas with deep canyons, buttes which may be numerous or scattered, or simple escarpments may be encountered. In a few places igneous intrusions are seen as dikes or as basaltic layers capping large mesas (true mesas in this case). From these features the mountain-front zone derives its varied character; the mountain upland on the west, and the plains extending far to the east, are of less irregular structure.

Arrangement of the component ranges and smaller ridges *en echelon* is a notable feature of the easternmost line of mountains. Ranges which are in general north and south of each other are themselves oriented with the northern end a little to the west. MARVINE writes (7, p. 132):

[1] The distribution of the remnants of the High Plains may be seen in a map by JOHNSON in the article mentioned.

[2] A true mesa is a tableland capped by a more resistant stratum which keeps the top flat by retarding erosion except on the sides. The débris-covered terraces flanking the mountains are like a true mesa in that the rock-waste layer acts as a more resistant cap.

In traveling from the north along the zone of hogbacks lying at the base of the mountains southward, the traveler finds the mountain-slope directly west of him falling lower and lower until it becomes an insignificant ridge, and finally dies away in the plains.

Passing around the southern end of the diminishing ridge the main mountain-slope is found lying several miles to the west, and separated from the ridge by a baylike valley extending northward behind it. . . . . The ridges are uplifted or anticlinal folds, the valleys depressed or synclinal folds, both dying away southward into the flatness of the plains.

The minor embayments due to echelon arrangement may be made out only in a large scale map, but the major embayments at the south end of the Rampart Range, the Pike's Peak highland, and the Greenhorn Mountains can easily be seen in fig. 1.

A more detailed view of the typical land-forms and vegetation-forms encountered in passing from mountains to plains traverses the several north-south zones in the following order: first the granitic foothills; then the transition zone of the mountain-front, with its upturned ridges, its mesas and graded slopes, and in places its plateau areas, buttes, and escarpments; and lastly the plains themselves.

GRANITIC FOOTHILLS

The mountain plateau is in most places submaturely dissected, the original upland level being represented only by the rounded tops of the hills (fig. 3). Slopes and summits are thinly covered with rock-waste. Occasional resistant dikes and ledges give craggy exposures of massive rock, not covered by any soil or débris. Below these, or on the sides of steeper ravines, are talus slopes of variously sized rocks, or slides of "granite-gravel."[3] Table I is a synopsis of topographic areas of the foothills arranged as habitats, and, correlated with these, the characteristic vegetation-types. Edaphic conditions largely determined by topography (local position in relation to surroundings, direction, amount of slope, and soil texture) have been discussed in the account of foothills vegetation (18).

This two-column form of presentation is adopted as being concise, as emphasizing relations between physiographic and ontographic features (the environment and the environed), and as permitting a more comprehensive view of the whole complex and its

[3] Decomposed granite in small angular fragments.

parts than can be obtained by the linear arrangement. Geographers will note that topographic *areas* rather than *land-forms* are used as the units of area of physical conditions (habitats), since land-forms, such as mesas and ravines, may include several topographic areas presenting quite diverse environmental conditions. Moreover, a single topographic area, even if physically uniform, may allow the growth within it of several more or less distinct vegetation-types.

FIG. 3.—Maturely dissected foothills near Boulder Creek: pine-sprinkled, rather than forested, surface mostly covered with dry grassland.

A brief statement concerning mountain parks may be made. These are small plains or flat valleys shut in on all sides by hills. They are not well developed in the foothills as compared with the montane zone. They are mostly formed where one of the principal eastward flowing streams is joined by tributaries from valleys opening into the park. There is a single outlet. Many of the montane parks in the Front Range contain the terminal moraines of former valley glaciers from above, and their topography is in large measure the work of ice. The slight gradient causes many

TABLE I

TOPOGRAPHIC AREAS (HABITATS) AND ASSOCIATED VEGETATION-TYPES IN GRANITIC FOOTHILLS COMPLEX

TOPOGRAPHIC AREAS	VEGETATION-TYPES
The geographic mean is that presented by rather exposed and xerophytic sloping surfaces, thinly covered with rock-waste of mixed texture, rather gravelly and with surface rocks. Local departures from the general condition are as follows:	The general ground-cover is mixed foothills grassland and primitive grassland, largely of grasses and herbs of the plains, with admixture of Rocky Mountain herbs, not all xerophytic. Scattered rock pines and plants of the mixed shrub association, singly or in clumps, dot the surface. In special habitats occur:
1. Exposed rock surfaces (bowlders and rock-walls)	1. Xerophytic lichen association
2. Rock-crevices	2. *Selaginella*, shrubs of *Jamesia* and *Ribes*, rock pine
3. Rock-strewn detritus slopes	3. Mixed grassland, and mixed consocies of primitive grassland, with higher proportion of woody plants (rock pines, mixed shrub, *Ceanothus*, *Arctostaphylos*)
4. Rock-talus	4. *Artemisia frigida-Koeleria* consocies of primitive grassland (18)
5. Compacted granite-gravel floors and side-slopes	5. Compacted granite-gravel consocies of primitive grassland (18) with rosette plants; *Arctostaphylos*
6. Loose granite-gravel floors, washes, and talus (gravel-slides)	6. Primitive grassland, with *Geranium-Chrysopsis* consocies, mat (rosette) consocies of gravel-slides, etc.
7. Mixed-soil floors and detritus-slopes (fine soil with imbedded and superficial rock-fragments of various sizes)	7. Foothills mixed grassland, with addition of other components, *Ceanothus*, sumac, pine, etc.
8. Fine-soil floors and detritus-slopes (infrequent)	8. Foothills mixed grassland, of a form approaching plains short-grass
9. Less xerophytic side-slopes (mostly north-facing, mostly of considerable gradient, and best developed in valleys)	9. Mixture of mixed shrub, rather less xerophytic mixed grassland, and pine associations, with representatives of canyon forest and scattered trees of *Pseudotsuga*
10. Narrow mesophytic ravines (best developed as small side-canyons, especially on the south side of eastward flowing main streams)	10. Mesophytic representations of mixed shrub, *Pseudotsuga*, aspen, *Symphoricarpos*, canyon forest, and mesophytic grassland associations. Mosses, *Saxifraga*, etc., in wet rock-crevices
11. Stream-sides in shaded ravines	11. *Betula*, *Alnus*, *Corylus*, and *Acer glabrum* of the canyon forest; shrubs; moist-soil herbs, as *Heracleum*, *Rumex*, etc.
12. Stream-sides in open canyon bottoms	12. *Populus angustifolia*, willows, etc.

meanders and oxbows in the streams, and there are in some parks small lakes in morainal depressions. The stream-sides are frequently boggy, with meadows adjoining. The parks are mostly treeless, or nearly so, and show no signs of former or impending forestation. The exposed dry flats are covered with dry grassland, its composition depending on altitude and geographic position chiefly. Differences in soil texture cause local variation of the grassland, but this is less marked and less minutely local than on the hill slopes. Certain lower areas are occupied by meadow and sedge communities, and the rolling surfaces of moraines (in montane parks) are variable in soil texture, soil moisture, and in the composition of their grassland cover; but the greater part of park floors is well drained, flat, and quite uniformly covered with dry grassland. This vegetation, in any one park, forms what might be called a crystallization of the grassland of the neighboring hills, whether in foothills or montane zone, in view of the comparative uniformity of the grassland of the flats as contrasted with that of the diversified slopes of hill topography. The lower parks have a grassland cover very like that of coarse soil in the mountain-front area or in the plains (see description of Estes Park in the regional section). The higher parks have fewer plants of the plains and more of the mountains. There is a floristic and vegetational gradation from plains grassland through the lower parks to montane grassland as seen in the higher levels. The parks thus show a steplike series of floristic and ecological changes with altitude. RAMALEY (10, 11) for some years has studied park vegetation, especially in Boulder Park at Tolland, Colorado, on South Boulder Creek.

TRANSITION AREA OR MOUNTAIN-FRONT ZONE

The sedimentary rocks, lying upon the granite, are upturned at the monoclinal fold, and are seen in a horizontal series of exposures of strata, the lower and older members abutting on the granitic foothills to the west, the upper formations outcropping in order toward the east. Since the tilting at the mountain-front is for considerable distances greater than 45° (locally reaching 90° and even more, resulting in overturns), the lower formations have narrower zones of outcrop than the upper strata, which dip so slightly as to cover areas many miles wide in the plains. The

narrow zone of older and lower strata contains alternating resistant and soft members, giving rise to the hogback ridges and intervening valleys already mentioned, while the newer rocks are mostly soft shales and sandstones, giving a flat or rolling topography over the surface of the plains, with occasional escarpments at the edges of stream-valleys. Both angle of dip and hardness of rock, therefore, contribute to a differentiation, in the sedimentary area outside the foothills, of a relatively narrow ridge-valley mountain-front zone from the very broad and mostly flat plains region.

Just outside the ridge-and-valley zone is the graded slope to the plains, covered with rock-débris and dissected into terraces or mesas of varying level. In places along the mountain-front the ridge-and-valley topography is absent or poorly developed, either because the troughs are not yet carved beneath the slope from the granitic hills, or because the ridges are already planed (locally) to a graded floor. The terraces are also missing from certain parts of the mountain-front. The topographic complexes of the ridge country and of the mesa country may now be described separately.

THE HOGBACK RIDGES (CUESTAS) AND INTERVENING TROUGHS (figs. 4, 5).—Two of the numerous sedimentary strata overlying the crystalline rocks are so resistant as to form ridges over great lengths of the mountain-front. These two strata are of such conspicuous geographic importance that they merit distinctive names and since many persons know them by their geological names, these will be used here in a geographic capacity. The *Fountain* sandstone, which in most places lies directly upon the granites, is very thick, and is composed of dark red, rough arkose materials, variable in texture. It is in places more resistant than the granites, so that side-gulches tributary to the east-flowing streams of the foothills are common in the granite just beneath the Fountain. Continuous troughs between the Fountain and the granite are not frequent. In many places the hard red sandstones form broad smooth-faced crags lying upon the outer foothill slope, reaching maximum size in the well known "flat-irons" south of Boulder (fig. 6). The other hard stratum is the massive gray sandstone known as the *Dakota*. It is separated from the Fountain by several less resistant strata of considerable aggregate thickness,

FIGS. 4, 5.—Upturned sedimentary ridges of mountain-front zone: fig. 4, eastward view in Perry Park, where a broad flat valley has been leveled between ridges and outer granitic foothills; floor of flat is of compacted angular fragments; vegetation is primitive grassland alternating with scrub oak; Dawson Butte in far background; fig. 5, southward view, between Golden and Morrison, of longitudinal valley inside Dakota hogback, shown on left in long curve.

and is usually seen as a bold ridge parallel to the outer slope of the foothills some distance to the east. The term "hogback" is familiarly applied to the steep Dakota cuesta.

A deep and wide trough usually extends between the Fountain crags and the Dakota cuesta. The upper part of the east-facing slope of this trough is the outcrop of a "creamy sandstone," which in places forms prominent outcrops, or even strong ridges, as at Morrison at the mouth of Bear Creek. Just east of and below the creamy sandstone is an easily eroded shale, which gives its rich red color to the deep soil of the valley. The west-facing slope, below the Dakota crest, is the outcrop of a calcareous sandstone stratum which is weathered so slowly as to be covered only by a thin soil. In certain places this limy sandstone stratum is hard enough to form a separate ridge or hogback crest.

The Dakota hogback is one of the most constant and conspicuous topographic features of the mountain-front, since it is practically everywhere harder than the strata above and below. Its top is usually quite even and straight, representing the level of a former graded surface. Its crest is quite rocky; there is no soil except in the crevices.

The present graded slope to the plains begins usually with the outer slope of the Dakota hogback, through first a layer of dark shales, then a thin limestone overlaid by soft light-colored shales, then clays and shales. Near every east-flowing stream, however, the graded slope is likely to be cut beneath by side-gulches cutting down into the dark shales, leaving a cut-off mesa with the limestone at its high western end.

Local distribution of vegetation in the mountain-front belt of upturned sedimentary rocks presents a variability apparently dependent almost entirely upon topography and soil texture, just as in the area of granitic foothills. There seem to be few if any perceptible differences in the floras of the different geological formations which can be traced to chemical differences in the substratum. It is perhaps true that cedars are more frequent in the limestone or calcareous sands of the stratum just below the Dakota, where these are exposed in gulches which notch the Dakota hogbacks, and that there are certain slight floristic differences between

granitic and sedimentary areas. This question has been discussed by RAMALEY (9), who found the two areas about the same in floras (in the Poudre mountain-front area), with *Cercocarpus* abundantly represented in the sandstone but not in granite, *Selaginella* apparently absent from the sandstone, and lichens infrequent there. Following a suggestion from COWLES, it appears to the writer that differences in rate of erosion of the substratum may explain the distribution of lichens, and perhaps *Selaginella* also. The sandstones are rather soft in the Poudre area, and wear away too rapidly for the lichens to establish themselves abundantly. The Fountain sandstone is harder in the Boulder region than elsewhere, and there at least it bears lichens almost as abundantly as do the granites. *Selaginella* is frequent in the sedimentary rocks in the Boulder area, as RAMALEY has pointed out. The writer knows of no plants which are restricted to either sedimentary or granitic areas, the only observed differences being those of relative abundance. The gulches, exposed slopes and crests, etc., of the sedimentary area are quite comparable to similar topographic situations of the granitic foothills, and have practically the same plant assemblages.

The rocky upper slopes of the Fountain, the Dakota, and other ridge-making strata, where they occur, lack soil except in crevices, and are mostly bare, except where rock pines or pinyons, shrubs of rocky situations (*Cercocarpus*, *Ribes*, *Jamesia*, etc.), and crevice plants, including many xerophytic herbs, can obtain a foothold. The west slope of hogbacks is blufflike, usually, and rocky, while the east slope is less steep (depending on the local angle of dip) and likely to be strewn with débris, as are the slopes of the harder exposures of the valley, and these have shrubby or herbaceous vegetation, sparse, and of species of rocky situations. The softer shales occupying the bottom of the valley are usually deeply buried by débris (of fine soil with imbedded rock fragments of all sizes), and support a grassland vegetation, which is luxuriant in the rainier parts of the growing season and very dry the rest of the time. A stream-bed in the bottom of the valley may be bordered by a strip of mixed shrub, *Crataegus*, oak, or canyon forest; or if dry, by scattered narrow-leaf cottonwoods and willows. Mesophytic

ravines developing in the sedimentary area support mixed-shrub, woodland, or mesophytic herbaceous growths, as in the granitic foothills. Local meadows (mesophytic grassland) are found on slopes where seepage or a high water table moistens a deep soil for at least part of the growing season.

In places the sedimentary rocks have been worn down more than is common, so that they are mostly or in part reduced to a general grade, above which the more resistant layers rise locally. This is the condition in the valleys of some of the larger streams from the foothills, and is seen at Platte Canyon, partially at Bear Creek (Morrison), and also in Perry Park (fig. 4) and the Garden of the Gods. The floor of this graded surface, especially in the Fountain exposures, is likely to be covered very thinly with small angular fragments, loose or compacted. The vegetation, as well as the soil, is very like that of gravelly floors in the foothills, being a variant of the primitive grassland association, with scattered rosette or mat plants, *Bouteloua hirsuta*, etc.

The climatic transition in the zone of upturned sedimentary strata is rapid. At Boulder and elsewhere dense cloud-banks have frequently been seen to descend to or just beneath the Fountain crags without continuing outward and downward to the plains (figs. 6, 7). The outer granitic hills and upper sedimentary slopes receive greater and more frequent precipitation than the lower slopes and adjacent mesas and plains; it may rain slightly below while it snows considerably above (cf. fig. 12); the outer and lower slopes are more exposed to wind, less cloudy, and in places less shaded from the afternoon sun by the higher granitic hills than the inner valleys and upper slopes. No exact data are available for this sudden climatic transition. Where the outcrop of sedimentary ridges and valleys is wide, as in the northern mountain-front region, the outer hogbacks are severely exposed to sun and wind, as in the open plains. Their coarse rocky soil favors woody plants; the xerophytic *Cercocarpus* shrub assemblage is here more extensively developed than anywhere else.

Mesas and graded slopes of the débris-apron (fig. 8).—The general character of the graded slopes and their mesa-fragments has already been suggested. The mesas are of varying ages and

levels. They are described in the accounts of LEE (6), JOHNSON (5), FENNEMAN (2), SHANTZ (15), RAMALEY, ROBBINS, and DODDS (12), and VESTAL (17). The topographic parts of a mesa are: (1) the

FIGS. 6, 7.—Climatic transition at mountain-front: fig. 6, outer mountains just south of Boulder, seen from university campus; clouds beginning to form at summit of Green Mountain, while much of South Boulder Peak, at extreme left, is already obscured; snow covers the mountain slopes and fades out toward base of high mesas; roofs of distant buildings also white; fig. 7, practically same view, a little later, with upper slopes obscured; at one time it began to snow on mountains and upper mesas, and a few minutes later to rain in town; shortly afterward it changed to snow in the upper edge of town, so that the roof of the building with the short steeple at the right in midground, and of nearer houses, were well whitened, while rain still fell on the campus, less than half a mile away, and not more than 50 ft. lower; difference in elevation at mountain-front is critical as regards climatic change.

mesa-top, with flat surface covered with mixed rock-débris; (2) the edge or mesa-crest; (3) the side-slope; and (4) valleys or draws in the side-slope. The soil conditions and their effects on plant distribution have been discussed in the three articles last cited.

The débris-cover, where it has not been removed by recent erosion, extends far out into the plains. Its removal from the extensive areas of soft shales and clays marks a change from the flat terrace level to the easily eroded, gently rolling surface of much of the plains. The High Plains are extensive remnants of the old graded surface, away from the mountains.

The north-south distribution of the terraces is practically that of the mountain-front, although as conspicuous topographic forms the mesas are not so extensive. So far as effects on distribution of vegetation are concerned, the presence of the coarse mixed soil of

FIG. 8.—Table Mesa, about 7 miles north of Boulder; outlines of hills sketched in with ink; ridge DDD is Dakota hogback; Boulder mesas may be seen in figs. 6 and 7.

the detrital surfaces is the important physical condition. It permits the growth in the same small area of a rich variety of plants, representing numerous vegetation-types and different geographic elements.

PLATEAU AREAS, BUTTES, AND ESCARPMENTS (figs. 9–11).—Where the sedimentary strata are horizontal or of rather slight dip the harder layers protect the softer rocks beneath, and extensive plateau surfaces are left above the grade established by present erosion. These can be invaded only at the edge and by ravines which eat their way headward into the bluffs. Smaller elevated areas or buttes, recently or long ago cut away from plateaus by

meeting of two such ravines, are common. Older buttes are fewer and more distant from one another.

FIGS. 9, 10.—Buttes and plateau areas: fig. 9, North Table Mountain at Golden, west of Denver; this and South Table Mountain are capped with basalt; fig. 10, Fisher Peak, northern end of Raton mesas, as seen from valley of Purgatoire River, a few miles above Trinidad; upland in midground belongs to southern sedimentary plateau; vegetation is principally dry grassland with scattered pinyons and cedars and infrequent clumps of scrub oak.

The plateaus and buttes are found outside of the upturned ridge and valley zone wherever the surface rocks are rather resistant. These resistant strata are usually the most recent and uppermost,

although the much older Dakota is at the surface over considerable areas in the plains drained by the southern tributaries of the Arkansas. For a considerable thickness above the Dakota the strata are mostly soft shales, which erode too readily to give table-land topography.

The larger through streams and their tributaries have cut below the level of the High Plains, leaving escarpments which are particularly notable near the Platte-Arkansas divide. Plum and

FIG. 11.—Buttes and plateau areas: divide between East Plum and West Plum creeks, in Castle Rock area, showing some of rhyolite buttes; one of the most imposing of these, Dawson Butte, shown in fig. 4.

Cherry creeks, running north into the Platte from the divide, and Monument, running into Fountain Creek, south to the Arkansas, have eroded deep valleys parallel to the mountain-front. Away from the mountain-front proper these valleys are bounded by lines of steep cliffs, but the west border of Monument and West Plum Creek valleys is the graded slope from the foothills, with its débris-covered terraces. Isolated buttes are present within these valleys, some of them protected by caps of igneous rocks from local outflows.

The southern part of the Sangre de Cristo Range (sometimes considered as a separate mountain chain, the Culebra Range) is flanked on the east by a sedimentary plateau which rises abruptly above the plains in a steep line of bluffs. The plateau is of sandstones mostly, of slight dip, and is much dissected by the eastward

flowing streams and their tributaries. On it rests the highland of the Spanish Peaks, and it is ribbed by resistant dikes of igneous material from two outflows, one set radiating downward from the peaks themselves. With the plateaus should be classed the high lava-capped mesas of the mountain-front and plains in the area near the Colorado–New Mexico boundary.

As in the hogback ridges, vegetation distribution in the plateau and butte areas is largely determined by soil texture and topography. Atmospheric conditions vary with exposure to wind and sun. The tops of the plateaus are covered with short-grass and

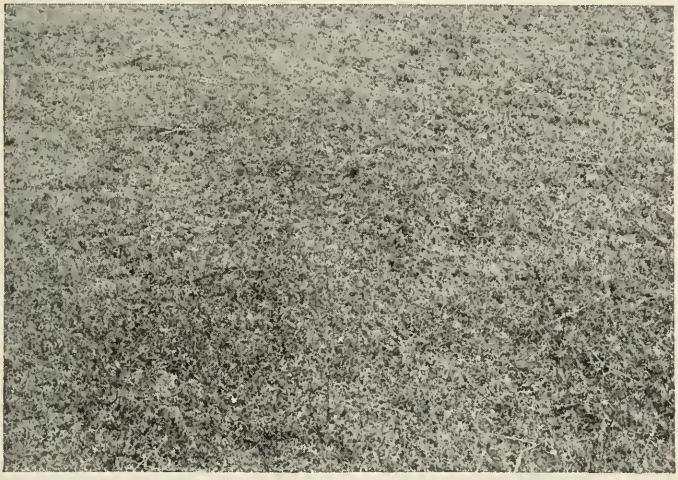


FIG. 12.—Unbroken short-grass ground cover in plains

mixed grassland over the level upland stretches of comparatively fine-textured soil. Exposed cliffs and crests, and rocky débris-slopes, afford lodging places for woody xerophytes (*Cercocarpus*, rock pines, pinyons, and cedars), with primitive grassland as the general ground-cover. The deeper and shaded parts of canyons and ravines approach a mesophytic condition, with mixed shrub and woodland vegetation.

PLAINS

Plains topography is typically flat or gently rolling country, with fine clay soil from a soft-shale substratum. Short-grass is the characteristic vegetation (fig. 12). Where the substratum is

sandstone the soil is more porous, with much sand; and plants of an assemblage typical of sandy soil are seen (17). Sand hills are present locally, usually to the leeward of larger streams.

Near the mountains the débris-cover, if present, considerably modifies topography, soil conditions, and vegetation. It may extend a long way into the plains, or may have been removed very near the beginning of the graded slope from the foothills.

Saline or alkaline areas are locally present. The valleys of the Arkansas and its tributaries (wet-weather streams, many of them, with trenched flood-channels) are in many places alkaline, and show prominent stands of *Sarcobatus-Chrysothamnus* vegetation.

Woody vegetation from the foothills extends locally far into the plains in rock outcrops, and along stony crests of stream-bluffs or terraces. The larger streams are bordered for many miles from the mountains by cottonwoods, usually scattered.

Climate

The region has a continental climate, semi-arid, less so at the base of the mountains and in the foothills, with most of the rainfall in the warmer months. Wind movement, proportion of sunshine, and evaporating power of the air are high in the plains, with wide extremes of temperature; all of these features are less marked in the foothills.

The southern part of the region is warmer and drier than the northern, and with different distribution of rainfall. The rapid east-west change in elevation and topography at and near the mountain-front is accompanied by more or less considerable climatic variation; this with the local peculiarities occasioned by the elevated Platte-Arkansas divide, and the differences between areas north and south of the divide, may be seen in the summaries of climatic data for the particular subregions. These data have been taken from the summary of Climatological Data for eastern Colorado, southeastern Wyoming, and northeastern Colorado.[4] The facts shown in table II should be considered in the light of their

[4] Section 6, northeastern New Mexico, by C. E. LINNEY. Section 7, region drained by the Arkansas in Colorado, and section 8, region drained by the Platte in Colorado, by F. H. BRANDENBURG. Section 24, southeastern Wyoming, by W. S. PALMER.

determinative influence upon the vegetation; this can be done in only the barest manner in this section, but these relations are again brought out in the part on geographic relations of the vegetation.

Temperature conditions of the different parts of the region may be summarized as follows: The foothills have a lower mean temperature and shorter period without frost than either plains or mountain-front. Certain of the foothills vegetation-types and

TABLE II

TEMPERATURE DATA

Area	Average mean temperature ° F.	Maximum temperature	Minimum temperature	Average number of days in growing season
Foothills (4)	43.1	100	−36	99
Northern (2)	42.0	98	−32	95
Southern (2)	44.3	100	−36	104
Mountain-front (5, excl. Divide)	50.5	104	−30	154
Northern (1, Boulder)	50.9	97	−20	164
Divide (2)	46.7	99	−33	122
Southern (4)	50.3	104	−30	151
Plains near mountains (5)	47.7	105	−38	138
Northern (3)	47.1	105	−38	134
Southern (2)	48.6	103	−32	143
Dry plains (5)	50.4	106	−45	151
Northern (2)	48.6	103	−45	145
Southern (3)	51.0	106	−32	156
"Northern area" (8)	45.8	105	−45	131
"Southern area" (11)	49.3	106	−36	142

The number of stations for each area is given in parentheses. The mountain-front does not include the two stations of the Platte-Arkansas divide, which is so much more elevated than other parts of the mountain-front as to be much cooler. The "northern and southern areas" are respectively the northern and southern parts of the region, each extending over foothills, mountain-front, and plains.

many of the plant species are characteristic of northeastern and northwestern coniferous forest regions, are in fact southern extensions of them. The boreal character is much more evident in the higher mountains than in the foothills.

The mountain-front has the longest frostless season, the highest mean temperature, the mildest winters, and the least range in temperature extremes. Mountain-front localities are mostly comparatively sheltered; temperature inversion is common. Early spring plants flower several weeks earlier at the mountain-front than in either plains or foothills; at Boulder in spring the

season is in general 2–3 weeks in advance of that of Denver, 14 miles from the mountains.

The divide between Platte and Arkansas drainage, which should be considered in connection with the mountain-front area, has a mean temperature and frostless period intermediate between those of mountain-front and foothills areas, as it is intermediate in altitude and in vegetation.

The plains have a slightly lower mean temperature and shorter season without frost than the mountain-front area; the temperature of the dry plains at some distance from the mountains approaches that of the mountain-front more closely than that of the plains adjoining it. This difference is accompanied by a floristic one. Temperature extremes are greatest in the plains, a condition inimical to growth of woody plants.

The plains, mountain-front, and foothills in northern Colorado ("northern area") are cooler than those to the south, but the north-south differences in temperature and length of growing season due to latitude are of much smaller range and influence upon vegetation than the east-west differences due to altitude and changes of topographic character.

For purposes of comparison table III includes rainfall data for the higher parts of the mountains bordering the foothills on the west (montane zone), and for the plains of eastern Colorado bordering the region studied on the east. Annual rainfall is higher to the west, increasing with elevation, and higher also in the eastern plains, as a part of the gradual geographic increase of rainfall from the dry belt of the Great Plains eastward through the prairie region to the border of the eastern deciduous forest region. The eastern plains mark the transition from short-grass plains to the taller prairie-grass vegetation of the prairie, and are known in Colorado as "the rain belt." The driest part of the plains region lies between the rain belt and the plains near the mountains, in a zone distant from the mountains about 18–25 miles, and of a breadth 30–60 miles. It is narrowed on the west by the elevation of the Platte-Arkansas divide, and extends farther eastward in the Arkansas River Valley. It extends only a little way north into Wyoming and apparently is much narrowed on the west in extreme southern

TABLE III

RAINFALL DATA

Area	Annual	January	February	March	April	May	June	July	August	September	October	November	December
Montane zone (6) 8250–10,265	**22.58**	0.80	1.10	1.96	2.75	2.85	2.01	3.38	2.70	1.61	1.64	0.80	0.99
Foothills (10) 6890–8000	**16.30**	0.42	0.60	1.11	2.17	2.23	1.27	2.79	2.06	1.29	1.27	0.55	0.44
Northern (6)	16.75	0.42	0.68	1.38	2.26	2.88	1.47	2.20	1.90	1.29	1.31	0.47	0.49
Southern (4)	15.63	0.44	0.49	0.69	2.03	1.25	1.18	3.67	2.29	1.27	1.23	0.68	0.37
Mountain-front (13) 5069–7373	**16.25**	0.40	0.68	1.07	2.06	2.59	1.79	2.41	1.81	1.23	1.11	0.53	0.54
Northern (5)	16.95	0.46	0.68	1.48	2.64	3.11	1.56	1.99	1.29	1.31	1.29	0.55	0.52
Divide (3)	16.53	0.35	0.56	0.94	2.08	2.69	2.17	2.73	1.96	0.77	1.11	0.47	0.69
Southern (5)	15.38	0.35	0.74	0.72	1.47	2.01	1.78	2.69	2.25	1.37	0.93	0.56	0.48
Plains near mountains (6) 4985–6098	**14.36**	0.33	0.49	0.81	1.85	2.28	1.76	2.17	1.81	1.20	0.81	0.47	0.43
Northern (3)	14.22	0.45	0.51	0.97	2.02	2.70	1.57	1.87	1.34	1.05	0.90	0.43	0.44
Southern (3)	14.54	0.20	0.47	0.65	1.67	1.86	1.94	2.47	2.27	1.34	0.72	0.51	0.43
"Northern area" (14)	**16.28**	0.44	0.64	1.33	2.20	2.92	1.53	2.06	1.57	1.27	1.22	0.50	0.49
"Southern area" (12)	**15.25**	0.35	0.59	0.69	1.46	1.72	1.56	2.96	2.27	1.33	0.93	0.59	0.43
Dry plains belt (7) 3899–5400	**12.73**	0.26	0.39	0.74	1.62	2.17	1.49	2.26	1.67	0.79	0.75	0.29	0.36
Eastern plains (8) 3380–5178	**16.95**	0.35	0.53	0.76	2.01	2.59	2.58	2.89	2.06	1.29	0.87	0.52	0.52

The northern area is the aggregate of northern foothills, mountain-front, and plains; the southern area is its southern equivalent. The number of stations is given in parentheses for each area, and the range of altitude above sea-level of the several north-south zones (in feet) is also included. The rainfall figures are in inches.

Colorado and northeastern New Mexico by the lava-capped plateaus which there extend eastward from the mountains.

As for the mountain-front and adjacent foothills and plains, the first two average about the same, the mountain-front receiving slightly more rainfall in the north and at the divide than the foothills. This may perhaps be due to the fact that in the northern part of the state, as at Boulder, the rain usually comes with east and northeast winds; and since the change of elevation is greatest at the mountain-front, more precipitation might occur there than in the foothills beyond. At Boulder the more mesophytic forms of vegetation occur more frequently and in larger areas in the sedimentary rocks of the mountain-front than in the granite foothills half a mile or a mile inside the foothills. In the southern part of the state the mountain-front is drier than the foothills, as a rule. The plains near the mountains receive almost 2 inches less rain, on the average, than foothills and mountain-front, and the dry plains to the east nearly another 2 inches less.

The "northern area" (foothills, mountain-front, and adjacent plains) receives on the average about 1 inch greater rainfall than the "southern area." Coupled with the higher temperature and greater evaporation, this results in a considerably more xerophytic vegetation south of the Platte-Arkansas divide. There is little difference in the plains, but at the mountain-front, with a difference of 1.57 inches, the vegetation to the south is markedly drier.

COOPER finds, in the chaparral region of California, that very slight differences in the original physical conditions of north and south slopes result in very marked differences in vegetation. The same principle seems to apply, in perhaps a smaller degree, in a semi-arid region like the Colorado mountain-front. It appears that differences in rainfall from place to place, or from month to month, although small in absolute amount, can be critical in their influence upon vegetation distribution. The slight differences appear to represent marginal values above or below a critical point. The difference in vegetation in two areas, moreover, is not necessarily the result of climatic difference, but is a resultant of differences in soil, topography, geographic position, and vegetational history, in addition to climate. It should not be surprising, therefore, that

areas having climates not widely dissimilar, as the plains of the rain belt and the northern foothills, should have distinctly unlike vegetation.

Minimum rainfall.—One factor which seems to be partly responsible for the generally xerophytic character of the entire region studied, the plains in particular, is the wide variation in the amount of rainfall from year to year. The minima have been

TABLE IV

Minimum annual rainfall

Area	Number of stations with records		Average minimum for area		Lowest minimum recorded for any station in area	
	1893	Other years	1893	Other years	1893	Other years
Montane zone.....	—	6	—	**15.65**	16.55 Frances	**11.36** (1907) Cripple Creek
Foothills.....	4	9	—	**12.83**	**7.16** Box Elder	10.93 (1908) Cheesman
Mountain-front and divide.....	7	11	9.12	**11.91**	**7.03** Waterdale	8.76 (1890) Table Rock
Plains near mountains.	5	6	9.39	**8.91**	7.11 Fort Collins	**5.04** (1876) Cheyenne
Dry plains belt.....	7	7	8.11	**7.01**	5.40 Greeley	**3.78** (1894) Las Animas
Eastern plains.....	5	8	10.48	**10.61**	8.30 Cheyenne Wells	**6.97** (1894) Cope

Two stations within the foothills area are exceptional as to rainfall, and have not been included in the averages. These are Salida in the Arkansas Valley above the Royal Gorge, and Westcliffe in the Wet Mountain Valley. Similarly, Canyon City at the debouchure of the Arkansas, and Raton and Las Vegas in New Mexico, have been excluded from the mountain-front area. The stations with the lowest minima have been mentioned in the table. The lowest minimum in each area, whether in 1893 or in some other year, is printed in bold face. Except for Cheyenne Wells, which is remote from the mountains, all of the stations noted as having had least rainfall in 1893 are within a limited area (in the northern part of the region), which seems to have been most severely affected by the drought of that year.

tabulated for the several parts of the region from the climatic summaries of the Weather Bureau. The year 1893 happened to be exceptionally dry, and the minima for many of the stations fall in it. Dryness in other years has been of more local prevalence. It has seemed preferable to present the data for 1893 separately from that of other years. The data for 1893 are not available for all stations in each area, and so the number of stations from which data have been used is mentioned for each area (table IV). The column presenting the average minima for the several areas (minima

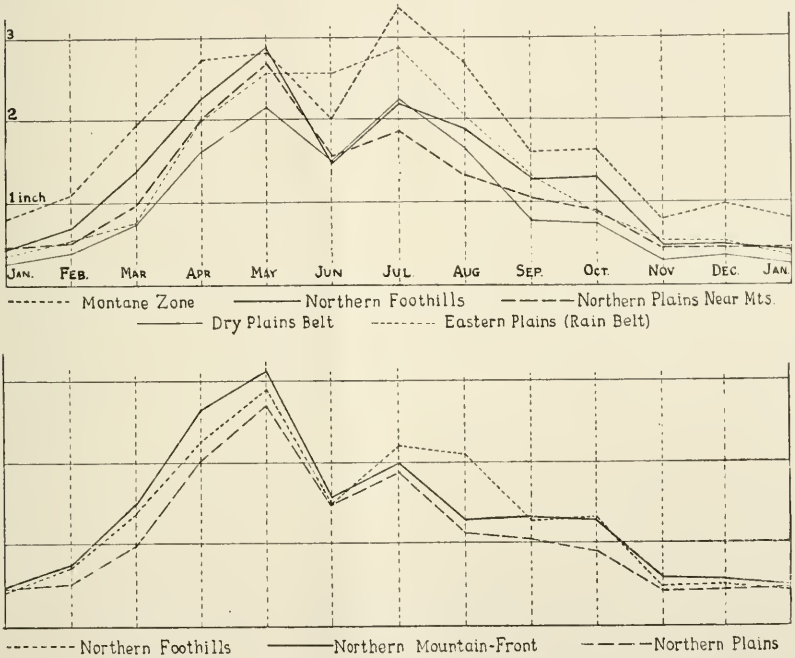
of all stations for each area averaged, excluding figures for 1893) seems to express the main fact of the table, that the rainfall reaches successively lower minima downward and eastward from the mountain zone through the foothills, mountain-front, and adjacent plains to the dry plains belt, beyond which the minima rise gradually with the gradual increase of rainfall eastward into the prairie-grass region. It appears also that annual rainfall values falling considerably below the average (as much as 4 inches below) occur more frequently in the plains than in the mountain-front and foothills areas. The well known uncertainty of farming without irrigation in much of eastern Colorado, due to frequency of very dry years, indicates further that it is not the average rainfall so much as the constantly recurring minimum which determines whether or not an area can support a cultivated or natural vegetation which is other than decidedly xerophytic.

Seasonal distribution of rainfall.—On the whole, precipitation during the cooler months is quite low; this is not so true of the montane area just to the west of and above the foothills. The summer rainfall is greater, but in most places distributed rather unevenly. June is thus drier than either May or July over practically the entire region. The northern area near the mountain-front receives more rain in the spring and early summer months, while the southern area receives more of its rain during late summer. This difference between north and south is of far-reaching influence upon the character and distribution of vegetation. The details of seasonal distribution of rainfall are shown in the table of averages of rainfall data, and in figs. 13–16.

The northern and southern parts of the zones at and near the mountain-front are so different as to rainfall that they cannot be incorporated in single graphs. The northern parts of the zones are selected, therefore, as the more typical. The graph for the mountain-front is omitted to avoid overcrowding, but it can be seen in fig. 14. Excluding the eastern plains, the zones have the same type of rainfall, with greatest abundance in May and July, and a decline in June. The zones are successively drier with decrease of elevation, and this is almost as true for particular months as it is for the entire year. The eastern plains have higher

summer rainfall than the plains near the mountains; the distribution is similar, except that June is as rainy as May.

The graphs for foothills and plains near the mountains are repeated in fig. 14. These zones and the mountain-front have maximum rainfall in May, with a sharp decline in June, followed by slightly greater rainfall in July. Despite its less elevated position, the mountain-front receives greater spring rainfall than the foothills.

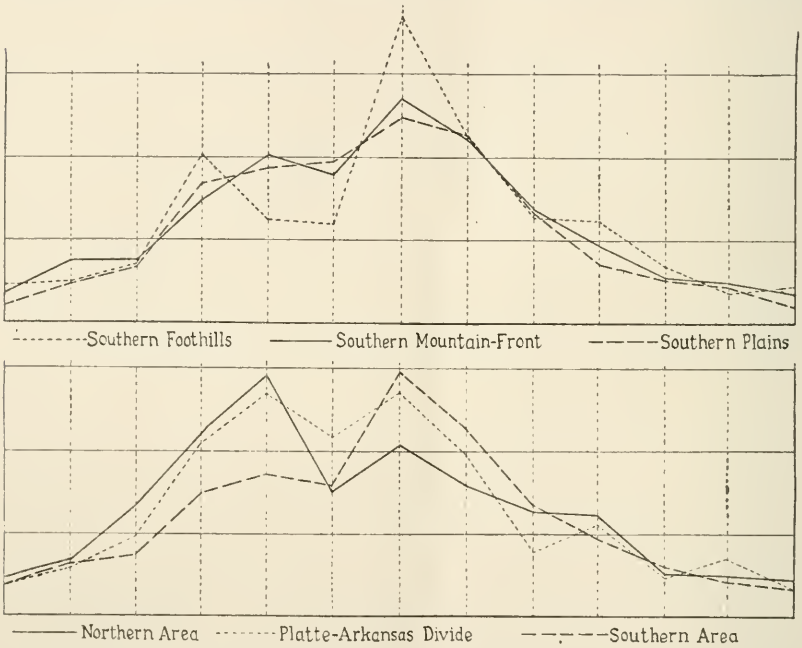


FIGS. 13, 14.—Seasonal distribution of rainfall: fig. 13, comparison of north-south zones; fig. 14, northern foothills, mountain-front, and plains.

The data for the south are not so dependable as for the north, for some of the few stations are exceptionally situated. The contrast shown with the northern area is marked, however. The rain is less abundant in spring and more abundant in July and August than to the north.

The graph shown for the "northern area" is a composite of the 3 in fig. 14, that of the "southern area" is a composite of those

in fig. 15; they contrast strongly. The northern area is characterized by the Rocky Mountain foothill type of rainfall, the southern area by the New Mexican type (WARD 19). Both of these types are described as having a single maximum, for the first in May, for the second in July-August. The northern area receives most of its rain from northeasterly winds; the southern area probably from southeasterly winds. The centrally situated Platte-Arkansas



FIGS. 15, 16.—Seasonal distribution of rainfall: fig. 15, southern foothills, mountain-front, and plains; fig. 16, northern and southern areas and divide.

divide receives rain from both directions, and has both maxima, with a higher June rainfall, partly because of its considerable elevation. Probably rain is carried from either direction past the divide, producing secondary maxima, in July in the northern area, and in April in the south.

The abundant rainfall of the divide, especially in June, forms a local rainfall type which is intermediate between that of well watered parts of the foothill zone and that of the eastern or rain-

belt plains. The divide is also cooler than most parts of the mountain-front and adjoining plains. The vegetation of the divide is likewise transitional between that of rain belt and foothills, with the more nearly mesophytic forms of grassland, and with woody plants of the foothills extending many miles eastward from the mountains.

The effects upon the vegetation of the difference in distribution of precipitation north and south of the divide are discussed in the section on geographic relations, but may be summarized briefly herewith.

TABLE V

INFLUENCE OF SEASONAL DISTRIBUTION OF RAINFALL ON VEGETATION

NORTHERN AREA	SOUTHERN AREA
The greatest rainfall is in April and May.	The greatest rainfall is in July and August.
There is greater activity of vegetation, more luxuriant growth, and greatest abundance of flowers in spring.	There is greater activity of vegetation and more luxuriant growth in late summer.
There are many spring-flowering plants from the mountains of rather mesophytic character, in mixture with plains plants in the mountain-front zone.	There is absence or scarcity of spring-flowering mountain plants, and greater prevalence of plains plants in the mountain-front zone.
Distribution of the bunch-grass association and of the less xerophytic plants, requiring a long season of continued moisture, is limited.	Distribution of bunch-grass is less restricted; there is a greater prevalence of late-flowering plants not intensely xerophytic, as some of the asters and goldenrods, etc.
The northern plains near the mountain-front flower luxuriantly in spring and early summer, but only the more xerophytic composites, etc., in late summer, in which respect the plains are more like the driest plains just east of them in late summer.	The southern plains near the mountains contain fewer spring flowers, but many long-season plants absent from the dry plains and the northern plains near the mountains are present, as the annual sunflowers. In this respect the plains are more like those of the rain belt of eastern Colorado in late summer.

It is remembered that the southern area is in general drier and warmer, with somewhat more xerophytic vegetation than the northern area, and that differences in vegetation due to this cause must be distinguished as well as possible from those due to different distribution of rainfall.

Evaporating power of the air has not been subject to geographic-statistical treatment, since there are no data. It was beyond the scope of the present study to have attempted instrumental investigation on a scale large enough to be of value. There seems to be little doubt that, as a geographic factor *in regions of continental climate*, evaporating power of the air is of about the same indicator value as rainfall. It varies geographically about as does rainfall, in inverse ratio, since evaporating power is, in large measure, a function of rainfall. This same inverse ratio seems to hold in seasonal distribution as well as geographically. This may be seen from the graphs of WEAVER (20), and from data obtained by COOPER in a study of chaparral in California.

As a local factor evaporation is separately treated in the discussion of local distribution of vegetation.

For further discussion of the climatology of Colorado in relation to vegetation the reader is referred to the articles of SHANTZ (15, 16), RAMALEY (12), and ROBBINS (13, 14). Data may be had from the bulletins of the United States Weather Bureau, Colorado College, the Agricultural Experiment Station at Fort Collins, and the Bureau of Plant Industry.

Local distribution of vegetation

PHYSICAL FACTORS.—Local physical conditions affecting plant distribution are those concerned with *substratum* and *soil;* with *topography*, especially *local position* with respect to surroundings, and *slope* of surface, as regards both steepness and direction of exposure; and with *local variation in atmospheric conditions*, as controlled primarily by topography. The variability of these factors within the region is great, and their interactions are complex. Descriptions of the soil, topography, atmospheric conditions, etc., of the different parts of the region are scattered through both systematic and regional sections of this study, and a lengthy discussion at this point would be out of place. A few references to other parts, and certain incidental comments, may here be made.

The character of the substratum, and some of its influences in determining soil conditions and topography, are indicated in the

account of the sedimentary area. The contrast between the granite soil of the foothills and the soil of sedimentary origin lying just outside, with its slight selective action on flora and vegetation, has also been noted. Other mentions of soils, especially as regards soil texture, are scattered.

Topography is systematically treated for particular regions and smaller areas by dividing each type of topographic complex into topographic areas or habitats; with each type is correlated the particular plant community or the several communities which accompany it. In the regional section will be found similar analyses of the cuesta, high mesa, mesa-terrace, and flood-plain complexes. Particular physical factors controlled by local position and by slope are mentioned in a former article (18).

Atmospheric factors vary locally in this region to a probably not very great extent, but even slight differences may be critical, as has been found by COOPER in the California chaparral. The factor of greatest influence upon plant life, and the one most readily measured, is the evaporating power of the air, the value of which represents the resultant of several contributing factors. Local distribution of evaporating power is believed to be controlled primarily by differences in topography, and secondarily by differences in vegetation-cover. That is to say, flatness of the land surface makes for comparative uniformity of exposure to wind and sun; hilliness causes diversity of exposure. Local water or wet-soil surfaces may lower evaporating power by contributing much water vapor to the air. Topography thus determines the *original* local distribution of evaporating power. This original local distribution is modified by vegetation-cover. In flat country the uniformity is changed. Low and open vegetation lowers evaporating power at the ground surface only slightly, but mesophytic closed forest lowers it very greatly (GATES 3, 4). In hilly country in not too humid climates the originally protected ravines and shaded or wind-sheltered slopes may develop mesophytic vegetation which still further lowers evaporation, while the originally exposed slopes and summits usually remain xerophytic. Thus, in the mountain-front region here considered, primary environmental differences

due to topography may rather thoroughly control vegetation distribution. In such cases the reaction of vegetation-cover upon local evaporation conditions may merely heighten the original topographically determined contrast between protected and exposed habitats. Topography governs local vegetation distribution through the mediative influence of a number of physical factors, of which evaporating power is one. Depending as it does upon several other factors, evaporation forms a convenient index of habitat, but is not in itself the basic controlling condition. For these reasons the writer has subordinated the influence of evaporating power upon local distribution to that of topography.

The sudden change of elevation at the mountain-front is a topographic condition affecting evaporating power. At many places the hogbacks, mesas, and outer slopes receive no direct sunlight during several hours before sunset, being shaded by the higher slopes immediately to the west. This contributes to the comparative mesophytism of certain mountain-front stations where the descent from foothills to plains is more than ordinarily abrupt.

Direction of exposure affects local atmospheric conditions and vegetation in many easily observed ways. Cloudiness and showers occur on summer afternoons much more frequently than in the mornings, as RAMALEY has noted. East-facing slopes are thus likely to be drier than west-facing slopes (the latter are less frequent east of the range-crest). As would be expected, the difference between north- and south-facing slopes is considerable, the latter being more exposed to sun and conditions favoring rapid evaporation, and with sparser, more xerophytic vegetation. In open parts of the foothills where slopes are quite gentle the north-facing slopes are not sufficiently sheltered from sun and wind to differ in vegetation from the south-facing slopes in any marked degree. Steep north slopes, or both sides of steep and narrow ravines which run down to the north, however, are quite mesophytic. In different parts of so large a territory the combinations of contrasting vegetation of north and south slopes would be expected to vary, and a few of them are listed herewith by way of illustration.

TABLE VI

EFFECTS OF DIRECTION OF SLOPE UPON LOCAL DISTRIBUTION

Locality	Vegetation of south-facing slope	Vegetation of north-facing slope
Poudre foothills	Grassland	Scattered rock pine, with more mesophytic vegetation infrequent
Foothills near Boulder	Grassland, rock pine, mixed shrub	*Pseudotsuga*, canyon forest, rock pine, mesophytic grassland
Poudre mountain-front	Grassland	*Cercocarpus*, with very scattered rock pines in rocky places; grassland in fine soil
Mountain-front near Boulder	Grassland, mostly	Grassland with rock pine, mixed shrub, etc.
South of Golden, mountain-front	Grassland and *Cercocarpus*	Grassland with scattered rock pine and mixed shrub
Perry Park	Oak and grassland	Rock pine and *Pseudotsuga*
Palmer Lake	Oak	*Pseudotsuga*
Southern mountain-front in general	Pinyon-cedar, dry grassland, and scattered oaks	Closer and taller oak growth with rock pines

FACTORS OTHER THAN PHYSICAL CONDITIONS OF HABITAT.—If the physical conditions which determine the habitat and all their interactions and variations were fully known, however, the local distribution of plant communities as observed would only partially be explained. Within even a very small part of the region studied correlations between physical habitats and vegetation-types must not be too closely drawn. The rock pine, for example, grows in any soil or on any slope; its presence or absence in any particular situation is not alone a matter of physical conditions there and then operative. Local distribution of vegetation-types in these partly unstable and locally very diverse situations depends also on at least three other sets of conditions: (1) range of toleration, in individual species or groups of species, of variation of physical conditions; (2) local historic factors, physical and vegetational, which have been operative in any given spot (these often cannot be determined); (3) accident of seed distribution and germination. For these reasons it seems best to characterize the vegetation-units, in most cases, from the vegetation itself rather than from habitat. There can be no question

that, in general, local variation of present physical conditions of the habitat governs to a considerable degree the distribution of plant communities, but the need of at least recognizing these other sets of factors should be emphasized. It must be further seen that, in the invasion of a new habitat, representatives from more than one plant community can be successful in establishing themselves, resulting in *mixed vegetation-types*. In fact, probably the greater part of the area studied is occupied by mixed associations or *mictia* (CLEMENTS). Even areas of established vegetation are usually open enough to permit the continual ecesis within them of new plant immigrants from quite different communities. This diversity is likely to be relatively enduring, for plant competition usually does not here operate to exclude all but a single type of dominants. The opposite relation between plants, which may be called accommodation, is as greatly in evidence. The control exerted by vegetation upon the physical environment is slight over the generally xerophytic mountain-front region.

A second factor contributing to the mixed effect is the frequent extremely local variability of physical conditions within the habitat. This might be called *mosaic variability*, and its effect a *mosaic mixture* of vegetation-types. The influence of large surface rocks partly imbedded in fine soil, allowing the growth of comparatively mesophytic plants in a rather constant interspersal with xerophytes over a considerable area, may be cited as an example.

Vegetation-types and their distribution

Since the plant communities have been described separately in the two articles preceding this, their systematic characterization here may be condensed very considerably. A tabular view of the communities, giving some idea of their general character and of their distribution in the main geographic divisions of the region studied, is shown in table VII.

Some of the more important features of the particular associations may now be noted. Details and references to other accounts may be found in the articles preceding. The general appearance of certain vegetation-types may be seen in fig. 17.

Lichen association.—Lichens, especially *Rinodina*, *Lecanora*, and *Parmelia conspersa*, partly cover the dry rock surfaces, especially granites in the foothills and the craggy outcrops and loose surface rocks of the mountain-front. Rock exposures are infrequent in the plains proper.

TABLE VII

CONSPECTUS OF ASSOCIATIONS

	Foothills	Mountain-front	Plains
Thallus vegetation	Lichen association	Lichen association	(Lichen association)
Grassland			
Extensive, climatic	Foothills grassland	Mixed short-grass	Short-grass
Local, edaphic			
Xerophytic	.....	Wheat-grass *Stipa-Aristida*	Wheat-grass *Stipa-Aristida*
Less xerophytic	Bunch-grass, plus a foothills element Mesophytic grassland forest herb type meadow type	Bunch-grass Prairie-grass meadow type mixed type	Bunch-grass (Local, infrequent, prairie-grass-like communities) (meadow type) (mixed type)
Primitive	Foothills primitive grassland *Artemisia frigida* consocies	Primitive grassland *Artemisia frigida* consocies Primitive bunch-grass	Primitive grassland *Artemisia-Gutierrezia* consocies Primitive bunch-grass
Shrub vegetation			
Xerophytic	*Cercocarpus* association	*Chrysothamnus-Sarcobatus* association *Cercocarpus* association	*Chrysothamnus-Sarcobatus* association
Xerophytic to mesophytic	Mixed shrub association *Arctostaphylos* *Ceanothus* association *Symphoricarpos*	Mixed shrub association *Symphoricarpos*	(Local shrub communities) (*Symphoricarpos*)
Tree vegetation			
Coniferous			
Xerophytic	Pinyon-cedar association	Pinyon-cedar association	
Less xerophytic	Rock pine association	Rock pine association	
Relatively mesophytic	*Pseudotsuga* association	(*Pseudotsuga* association)	
Deciduous			
Xerophytic to mesophytic	Oak association *Populus-Salix* association	Oak association *Populus-Salix* association	*Populus-Salix* association
Relatively mesophytic	Canyon forest (Aspen association)	Canyon forest	

Associations with equivalent or similar representation in plains, mountain-front, and foothills areas are shown on the same horizontal line. Very local or poorly developed representation of a community in a particular zone is indicated by parentheses.

Mixed grasslands and short-grass.—The shallow-rooted short-grasses, *Bouteloua* and *Bulbilis*, dominate the compacted fine soil surface of most of the plains, as the well known short-grass association. *Bouteloua* alone, with admixture of plants of different physiological and geographic character, is the important element of dry

FIG. 17.—Distribution of vegetation in typical northern mountain-front area (outlet of Gregory Canyon, near Boulder): habitats and vegetation-types distinguishable are: 1, craggy exposure of Fountain sandstone; 2, rock talus, with beginning of primitive grassland; 3, stabilized slope with primitive grassland; 4, mixed grassland, in places approaching mesophytic type, mostly on northwest-facing slope; 5, more nearly xerophytic mixed grassland, with bunch-grass and ruderal elements on northeast-facing slope; 6, *Symphoricarpos;* 7, mixed shrub; 8, canyon forest; 9, *Populus angustifolia* stream-side community; 10, rock pine; 11, *Pseudotsuga*, in scattered growth, including rock pine and other components, on north-facing slopes of Green Mountain

grassland in the débris-covered soil of the mesas and outwash-plains of the mountain-front (short-grass mixed association). The foothills mixed grassland, very similar to the mixed short-grass, is typical over the coarse surface of the granitic foothills.

Wheat-grass.—This taller but also shallow-rooted grass, *Agropyron Smithii*, dominates areas of loose clay in the mountain-front and plains. Its ecological character is not well understood.

Stipa-Aristida association.—These tufted xerophytic grasses of coarse soil occur frequently but not extensively, together or singly, with other rather deep-rooted plains xerophytes.

Bunch-grass.—Tufted perennial and deep-rooted grasses, depending on continued moisture, such as *Andropogon scoparius*, *A. furcatus*, *Sorghastrum nutans*, etc., are notable through most of the prairie region, almost absent in dry plains, but abundant in the rain belt of eastern Colorado; at the mountain-front and in the foothills, scatteringly in the north, but very frequent at the Platte-Arkansas divide and southward into New Mexico. In the foothills there are grasses of similar habit which mingle with the prairie bunch-grasses.

Mesophytic grasslands.—Mesophytic herbaceous growths are made up partly of prairie plants and partly of Rocky Mountain forest plants. The latter element is very considerable in occasional foothill ravines. Meadow growths of both foothills and mountain-front, in moist soil, with showy flowering plants like *Delphinium*, *Cerastium*, *Castilleja*, *Orthocarpus*, etc., are conspicuous in early summer, but not very frequent. The mountain-front in many places shows a mixed grassland much like that of eastern prairie, which has been called western prairie-grass. It has plants of the mixed short-grass, with components from bunch-grass and eastern prairie or forest border, with some foothills mesophytes, and a few plants characteristic of the mountain-front, like *Stipa viridula*.

Primitive grasslands.—Early stages of grassland developing in areas recently bared, or remaining for long in loose shifting slopes, are frequently seen. Prostrate plants with heavy taproots (rosette plants) are common. Gravel-slides in the foothills and dry stony crests of mesas, buttes, and ridges in the plains and mountain-front are the typical habitats. The *Bouteloua hirsuta*

and the *Artemisia frigida* consocies may be mentioned specially. The last is closely allied to the *Gutierrezia-Artemisia* association of the plains, very widespread, and continuing, at the expense of short-grass, with heavy grazing. In mountain-front and plains the primitive bunch-grass association, with *Panicum virgatum*, *Sporobolus cryptandrus*, *Stipa Vaseyi*, *Eriocoma*, etc., occupies sandy or loose-soil habitats recently disturbed.

Chrysothamnus-Sarcobatus association.—The shrubby composite, *Chrysothamnus* (rabbit-brush), and the chenopodiaceous greasewood occupy loose soil, mostly alkaline areas, on certain slopes in the mountain-front, and are particularly abundant in stream-bottoms in the southern plains.

Cercocarpus association.—Mountain-mahogany, of the rose family, is the only dominant in the open shrub growth of the mountain-front and outer foothills, in very dry exposed situations and usually stony soil. In the interstices between shrubs are plants of primitive grassland or mixed short-grass.

Mixed shrub association.—This is a heterogeneous assemblage of shrubs, ranging from xerophytic, like *Rhus trilobata*, to relatively mesophytic forms, like *Crataegus coloradensis*, in sheltered situations. The same species range through a variety of habitat conditions, and may form a community either as shrubs or trees. The mixed shrub grades into the canyon forest.

Arctostaphylos association.—The well known and widespread bearberry forms its characteristic mats in the foothills, mostly on compacted gravelly floors. It is more abundant in the upper foothills, in open places among the scattered pines. Its congener, *Juniperus communis sibirica*, is present but infrequent.

Ceanothus association.—*Ceanothus Fendleri* forms low matlike ground-cover in the lower foothills, similar to that of *Arctostaphylos*, though it is not evergreen, is of more southerly distribution, and ranges into drier and more exposed habitats. It favors the establishment of seedling mesophytes, and plays a part in revegetation of burned areas.

Symphoricarpos association.—The buckbrush, as it is called, occupies moist fine soil, and invades grassland in the mountain-front and foothills, as well as in the eastern prairie, in favorable

situations such as draws and seepage areas of slopes. It in turn is frequently displaced by taller woody vegetation.

Rock-pine association.—*Pinus scopulorum* is the important tree of the foothills. It ranges into very variable habitats, and is structurally variable in accordance. It forms infrequent close stands, but in most places is scattered, the ground between the trees being occupied by foothills mixed grassland, *Ceanothus*, *Arctostaphylos*, etc. It is frequent in rocky crests, etc., in the mountain-front, except in the south, where it is commonly replaced by pinyon. It extends very locally into the plains in broken country, on butte-crests, etc., and on the elevated Platte-Arkansas divide.

Pinyon-cedar association.—*Pinus edulis* and *Juniperus monosperma* are important xerophytic conifers of the southern mountain-front and lower foothills north to the Garden of the Gods, and extending into the southern plains on mesa-crests, canyon-walls, and bluffs of broad valleys. The soil is usually rocky or gravelly. The trees are low and rounded, and do not form a closed assemblage.

Pseudotsuga association.—*Pseudotsuga mucronata* forms the mesophytic or relatively mesophytic coniferous forest of the region, and is confined to sheltered ravines and steep north slopes in the foothills. It is infrequent at the mountain-front.

Oak association.—Small trees of the white-oak group, of uncertain taxonomic affinity, form dense copses or open woods in the lower foothills and in the mountain-front about as far north as Platte Canyon. In places grazing destroys the oak slowly and allows increase of grassland. The undergrowth of mesophytic oak areas is much like that of the canyon forest.

Populus-Salix association.—In stream-side areas of the foothills *Populus angustifolia* and 4 or 5 common willow species are frequent. Outside the mountains *Populus Sargentii*, and in the south *Populus Wislizeni*, replace the narrow-leaf cottonwood. Cottonwoods extend eastward into the plains for many miles along watercourses.

Canyon forest.—The deciduous trees of the foothill canyons and of ravines, etc., in the mountain-front, include *Alnus tenuifolia*, *Betula fontinalis* (these two common along mountain streams),

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Amelanchier alnifolia, *Prunus pennsylvanica*, *P. americana*, *P. demissa*, *Crataegus coloradensis* et spp., *Robinia neomexicana* (in the south only), *Acer glabrum*, and *A. Negundo*, with a few others. A few shrubs are present and a variable undergrowth, with one typical aspect of *Viola canadensis* Rybd., *Hydrophyllum*, and *Galium*. A *Ligusticum* is very abundant in places.

Aspen association.—*Populus tremuloides* is restricted, in all but the highest parts of the foothills, to relatively mesophytic ravines. It does not come up abundantly following burning of the pine forest, as is true in the higher elevations and farther north.

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POLYXYLIC STEM OF CYCAS MEDIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 252

(WITH ELEVEN FIGURES)

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(WITH ELEVEN FIGURES)

The question which lends particular interest to the polyxylic situation in 4 of the cycad genera (*Cycas*, *Macrozamia*, *Encephalartos*, and *Bowenia*) is whether or not the separate, concentric, vascular cylinders all originate with the differentiation of procambium strands in the plerome cylinder, and whether or not true protoxylem and protophloem are formed in every or in any instance. If protoxylem and protophloem are formed, are they or are they not orthodox in their detailed structures, and what is their condition in the older parts of the stem?

It is the purpose of this investigation to discover the exact origin and behavior of the different cambiums which develop the separate vascular cylinders, and to formulate some definite conclusions regarding the vascular elements which will leave the matter of this unusual method of secondary growth more clearly understood.

Historical

Although research among the cycads has been comparatively limited by lack of suitable material for study, several accounts have been published which deal more or less directly with the present problem. During an investigation of *Cycas siamensis* in 1885, CONSTANTIN and MOROT[1] concluded that the cambium of each supernumerary zone, laid down outside the first or normal cylinder, originated in the pericycle of the cylinder next inside.

In 1896 WORDSELL[2] published an account of the polyxylic stem of *Macrozamia*. In this particular genus he found the corti-

[1] CONSTANTIN, J., and MOROT, L., Sur l'origine des faisceaux libéro-ligneux supernuméraires dans la tige des Cycadées. Bull. Soc. Bot. France 32:173. 1885.

[2] WORDSELL, W. C., Anatomy of stem of *Macrozamia* compared with that of other genera of Cycadeae. Ann. Botany 10:601–620. 1896.

cal cylinders diminishing in the thickness of their wood and phloem as they neared the tip of the stem, until finally they disappeared entirely, the outermost disappearing first and the innermost last. Protoxylem, at least of the spiral kind, he found to be entirely obliterated in the normal cylinder, although it seems to have been of frequent occurrence in the leaf traces, where the crushing pressure of thickening cells was less effective. He mentions no protoxylem in connection with the supernumerary cylinders, either as to its presence or absence, nor in connection with leaf traces coming from these cylinders.

Scott's[3] work in 1897 led him to the conclusion that the polyxylic habit was a derivation from the habit of ancient stems among the Cycadofilicales which developed layers of concentric bundles, for example, *Medullosa*.

Wordsell[4] again in 1900 published an account of the seedling stem structure in *Bowenia*. There he found beginnings of a supernumerary vascular cylinder outside the normal one. Hints of concentric bundles, which he found in *Bowenia* and earlier in *Macrozamia*, led him to agree with Scott in the idea of the phylogenetic origin of supernumerary cylinders.

Coulter and Chamberlain[5] published in 1910 a summary of previous investigation pertaining to the vascular anatomy of cycad stems, and in addition gave a short description of the gross topography of the polyxylic habit.

Jeffrey's[6] work, published in 1917, is the most recent account of this cycad peculiarity. To him also it is apparent that supernumerary cylinders arise in the pericycle. He objects, however, to Scott's conclusions in regard to the phylogenetic origin of these vascular cylinders; he believes rather that they are a result of an ancient climbing habit of the stem. Such situations, he says, are frequent in numerous climbing stems of the present time, stems of both gymnosperms and dicotyledonous angiosperms.

[3] Scott, D. H., The anatomical characters presented by the peduncles of Cycadaceae. Ann. Botany 11:399–419. 1897.

[4] Wordsell, W. C., The anatomical structure of *Bowenia spectabilis*. Ann. Botany 14:159–160. 1900.

[5] Coulter, J. M., and Chamberlain, C. J., Morphology of gymnosperms. 1910.

[6] Jeffrey, E. C., The anatomy of woody plants. 1917.

The foregoing brief account of previous research, touching upon the problem at hand, gives a foundation upon which to work and from which to develop further lines of investigation.

Material and methods

Material used in the study of the problem was collected by Dr. CHAMBERLAIN near Rockhampton in Queensland, Australia. I take this occasion to express my appreciation of his generosity in giving up material from his own private collection for my study, and of his helpful suggestions during the investigation. The plant collected by CHAMBERLAIN was about 3 m. in height, as it occurred in nature, and bore at its tip a cluster of megasporophylls surrounded by a crown of foliage leaves. Two pieces were taken from the stem, one at the apex and one near the base. The former piece was the entire tip, including the upper 6 or 8 inches of the axis, together with foliage leaves and megasporophylls; the latter piece was a cross-section of the stem at a height of less than a foot above the soil, and was cut with a thickness of about 3 inches. Both pieces were put into formalin, where they have remained since the time of their collection in November 1911.

Pieces of the stem were thoroughly washed in water and then allowed to stand in 50 per cent hydrofluoric acid for a period of 4 weeks. Following this treatment, methods were employed which were based upon the fundamentals of technic as published by CHAMBERLAIN[7] in 1916. Such variations in these principles as were used grew out of the kindly suggestions of Miss LANGDON of this laboratory. To her my thanks are given for her valued assistance.

Investigation

GROSS TOPOGRAPHY

As would be expected, the pith of this specimen is relatively large. Its diameter at the stem base measures 5.3 cm., whereas the diameter of the entire stem at the base is only 20 cm. At the tip, where the gross diameter is 17.8 cm., the pith has a diam-

[7] CHAMBERLAIN, C. J., Methods of plant histology. 1916.

eter of 5.8 cm. There is practically no tapering to the columnar trunk excepting at the very apex, while the pith actually increases in diameter as it nears the apex, until it finally merges into the plerome cylinder (fig. 1).

At the base of the stem there are 3 separate and distinct vascular cylinders, the normal one nearest the pith and the 2 cortical ones developed concentrically about the normal. In previous accounts the first cortical cylinder has been reported to have a development of xylem and phloem equal to that of the normal cylinder, while the second and succeeding cortical cylinders diminish successively in that development toward the periphery of the stem. I find in this stem, however, that, near the base, the first cortical cylinder has a greater radial extent of xylem and phloem than has the normal one, while the second is about the equal of the normal, thus beginning the successive decrease of xylem and phloem development which would likely be continued further if other vascular cylinders were present (fig. 2). In each cylinder the xylem elements are of greater radial extent than phloem elements, the former occupying approximately three-fifths of the radial extent of the entire cylinder.

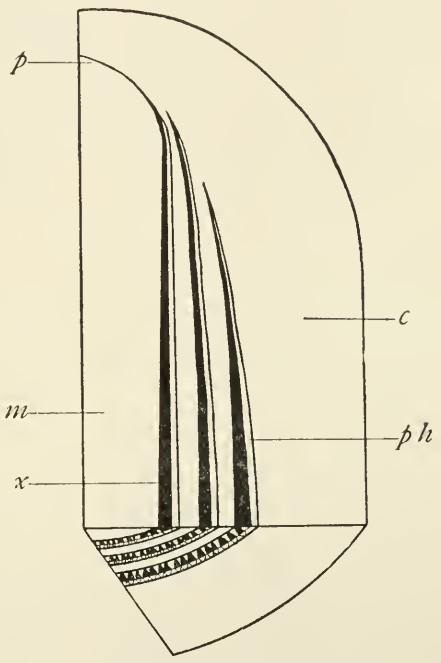

FIG. 1.—*Cycas media:* showing radial and transverse aspects of polyxylic stem near tip; *m*, pith; *p*, plerome; *x*, xylem; *ph*, phloem; *c*, cortex.

At the stem apex only 2 vascular cylinders are evident (fig. 3). Here the normal cylinder is seen to have xylem and phloem of slightly less radial extent than it has near the stem base, while

the first cortical cylinder has decreased so much in its dimensions that it is barely visible to the unaided eye. Furthermore, the latter occurs, not as a continuous cylinder, but rather as a cortical layer of separate, broad, and short bundles which are distinctly collateral. The outermost cortical cylinder entirely disappeared before reaching the height at which the section was taken. This

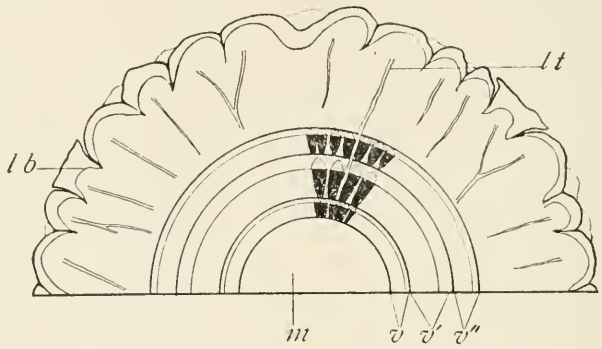

FIG. 2.—*Cycas media:* showing gross topography of transverse section of stem near base; *v*, *v'*, *v''*, 3 distinct vascular cylinders; *m*, pith; *lt*, leaf traces; *lb*, leaf bases.

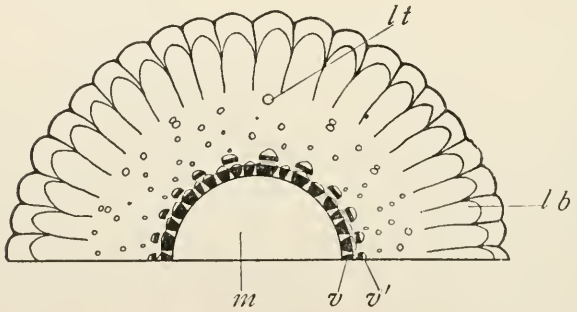

FIG. 3.— *Cycas media:* showing gross topography of transverse section of stem near tip; *v*, *v'*, 2 vascular cylinders; *m*, pith; *lt*, leaf traces; *lb*, leaf bases.

quite agrees with WORDSELL'S (*loc. cit.*) account of a situation exactly similar in *Macrozamia*. Fig. 1 represents the polyxylic structure diagrammatically, as it might be seen in radial section in the apical region of the stem. Differentiation which results in cortical cambium begins farther from the stem apex in each succeeding cylinder, being farthest in the outermost cylinder.

The cortex, true to cycadean character, is relatively large, as well as the pith. At the stem base the cortex measures 2.9 cm. between the outermost cortical cylinder and the leaf base, and at the tip 2 cm. A great many leaf traces traverse the cortex. At the base of the stem these traces are seen in longitudinal section (fig. 2), excepting at those places where they are just leaving the vascular cylinder. At the tip, however, leaf traces are invariably seen in transverse section (fig. 3), and they are characteristically double where they are about to enter a leaf base. Traces may leave any or all of the vascular cylinders, those from the inner ones passing to the cortex through the medullary rays of one or more outer cylinders.

DETAILED STRUCTURE

Normal cylinder.—Vascular bundles of the normal cylinder are long and narrow in transverse section (fig. 4), rarely becoming more than 3 or 4 cells in tangential thickness. Bundles taper to a rather sharp point toward the pith, and there is located the definite endarch protoxylem. Wordsell had difficulty in locating protoxylem in the stem of *Macrozamia* which he studied, for it had been obliterated by the crushing caused by thickening wood cells. In the specimen which I studied the protoxylem is still intact in the majority of cases, and is easily distinguished (fig. 4). Fig. 5 represents protoxylem of the normal cylinder, enlarged enough to show its detailed character. The cell walls are less thickened than those of the primary xylem above, and there are certainly no pits present, as there would be if the xylem were of secondary origin. In this particular instance pits are absent from the primary xylem also. This is an unusual condition, since, as in fig. 6, primary xylem of the normal cylinder is practically always scalariform. Fig. 6 shows the radial aspect of the normal cylinder in its centripetal region. Here protoxylem elements are unquestionably spiral in character, while the succeeding primary xylem is scalariform. It should be said here that spiral tracheids are of comparatively rare occurrence even in the normal cylinder; at least they are rare in stretches large enough to be correctly interpreted. The usual form of protoxylem is scalariform rather

than spiral. Since neither transverse nor radial preparations show crushed masses of cellular material at the centripetal ends of bundles, there can be no doubt that the xylem elements which can be seen to terminate the bundles are truly protoxylem, whether they are spiral or scalariform.[8]

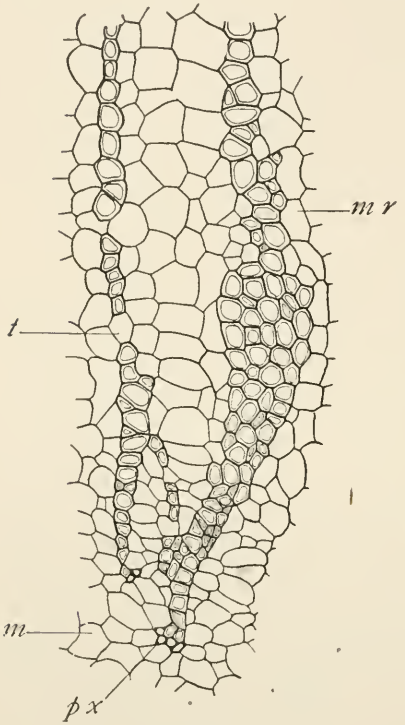

FIG. 4

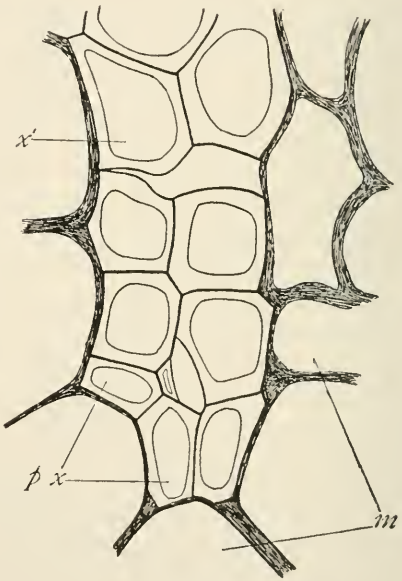

FIG. 5

FIGS. 4, 5.—*Cycas media:* fig. 4, transverse section of stem, showing only centripetal end of bundle of innermost cylinder; *m*, pith; *mr*, pith ray; *px*, distinct protoxylem; *t*, unthickened xylem elements; ×400; fig. 5, transverse section of stem, showing centripetal end of bundle of innermost cylinder highly magnified; *m*, pith; *px*, protoxylem; *x'*, primary xylem; pits apparent in none of these cells; ×850.

As in WORDSELL'S account, spiral tracheids here can be followed more easily in leaf traces off the normal cylinder than they can in the cylinder itself. The reason for this is not that these elements have been destroyed in the cylinder proper, but that

[8] CHAMBERLAIN, C. J., The adult cycad trunk. BOT. GAZ. 52:97. 1911.

they meander back and forth tangentially, so that only short patches can be caught here and there in a single radial section. The meandering habit is not so pronounced in the traces, and consequently longer stretches of primitive xylem elements can be seen and identified as such.

Secondary xylem of the normal cylinder is composed of tracheids which are characteristically pitted, the pits being confined largely to the radial walls, as described by both CHAMBERLAIN and JEFFREY.

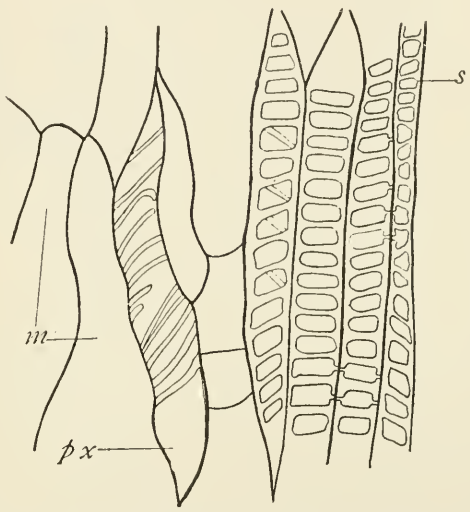

FIG. 6.—*Cycas media:* radial section of stem, showing centripetal end of bundle of innermost cylinder; *m*, pith; *px*, protoxylem distinctly spiral; *s*, scalariform tracheids of primary xylem, left one also having spiral thickenings; ×400.

The phloem situation of the normal cylinder adds emphasis to the fact of the latter's procambium origin, for protophloem is as distinct here as it is in any of the typical monoxylic cycad stems. Fig. 7 illustrates the upper phloem region of this cylinder, showing the crushed cellular substance which once was organized protophloem. This dark crushed mass has the appearance of a thick irregular ring in transverse section, entirely surrounding the normal cylinder and immediately inside the centripetal limits of the first cortical cylinder (fig. 8). The ring of course is interrupted here and there by medullary rays, but in many cases it extends unbroken across them, being squeezed in between the cells of the pith or cortical medulla. From this protophloem center primary and secondary phloem extend, fanlike, outward and downward in typical fashion. The rather startling character of the secondary phloem is its large number of suberized bast fibers compared to the number of sieve tubes. The former far

outnumber the latter, which occur here and there in short tangential rows between the bast.

First cortical cylinder.—The most intensive study of the cortical cylinder was made from preparations of the innermost one near the apex of the stem (fig. 3). Here details could be observed where the cylinder was in an early stage of development, and where its character could best be determined.

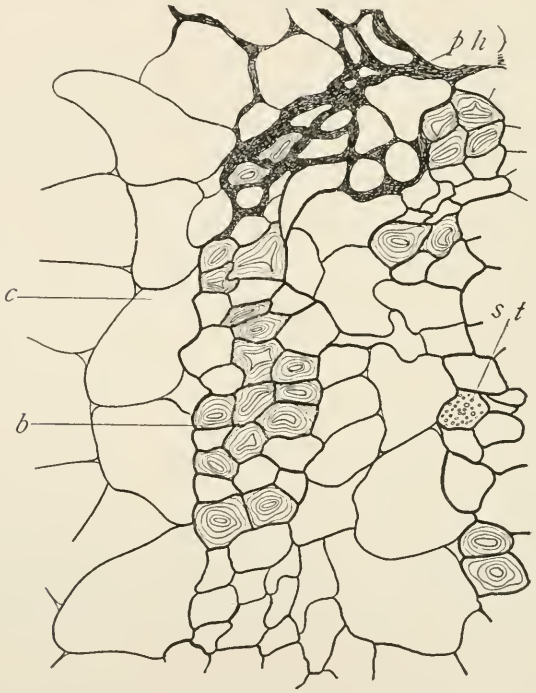

Fig. 7.—*Cycas media*: transverse section of stem, showing region of protophloem of innermost cylinder; *ph*, protophloem; *b*, suberized bast fibers of secondary phloem; *st*, sieve tube; *c*, cells of cortex; ×235.

Fig. 8 shows a transverse section of the entire cylinder, together with regions bordering it on both inner and outer boundaries. Apparently no protoxylem is present in these bundles. Cells at the centripetal limit of a bundle are not different from those nearer the cambium; there is little or no difference in size, shape, and thickness, in alignment, or in the character of cell

walls. Fig. 9 shows characteristics of the xylem in that region of the cylinder where protoxylem would be expected if there were any. All cells are uniformly thickened and, without exception, equipped with bordered pits, which is always a mark of secondary origin. In fig. 10 the same region is seen in radial section. Here the xylem element nearest the stem center, and even bordering on protophloem of the normal cylinder, is pitted. This one drawing illustrates tracheids of the first cortical cylinder which are as nearly scalariform as could be found; they are as rare as spiral tracheids are in the normal cylinder. By far the greater number of xylem elements in this centripetal region of the cylinder are pitted in exactly the same fashion as ordinary secondary tracheids of the normal cylinder, and they must in turn be considered as of secondary origin.

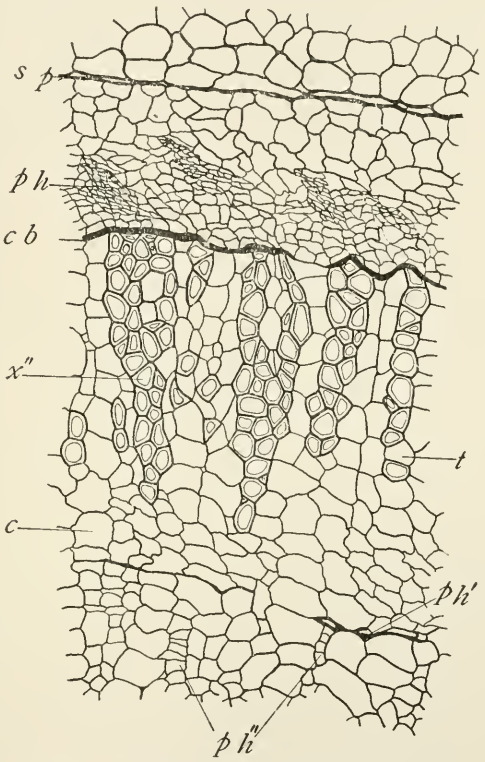

FIG. 8.—*Cycas media:* transverse section of stem, showing entire second cylinder as it appears near tip of stem; *ph′*, protophloem of first cylinder crushed; *ph″*, secondary phloem of first cylinder; *c*, cortical cells; *x″*, secondary xylem of second cylinder; *t*, unthickened xylem cells; *cb*, cambium; *ph*, secondary phloem of second cylinder; *sp*, split in cortex caused by expansion lower down of third cylinder; ×85.

In fig. 8 it will be seen that the region between the normal cylinder and the first cortical one is composed of purely cortical cells. Also the region between the first cortical cylinder and the split in the cortex, which marks the place where the second

cortical cylinder will appear, is purely cortical. No differentiation is evident between stelar pericycle and cortex; there is even no endodermis, and therefore there is no ground here for believing that the supernumerary cylinders originate in the pericycle. In view of Sister HELEN ANGELA'S[9] work with *Ceratozamia*, in which she found unquestionable cambiums at any place in the cortex from the stele to the periphery, it would be possible for the supernumerary cylinders of *Cycas* to originate in the cortex. In view of the evidence of fig. 8 it would also seem probable that the cylinders are truly cortical and not stelar.

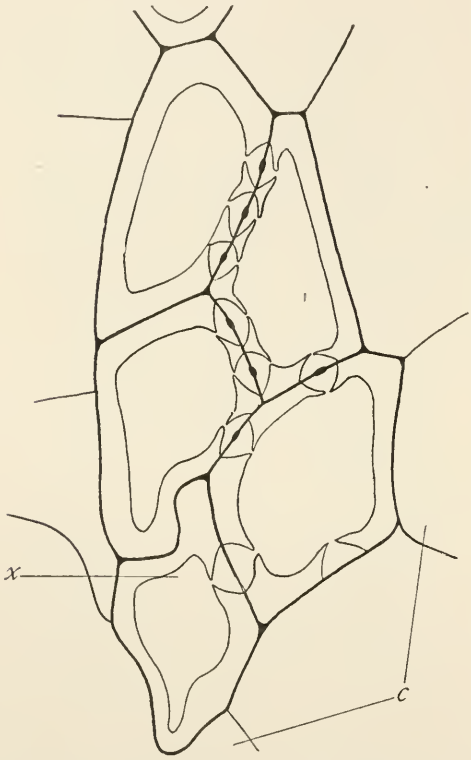

FIG. 9.—*Cycas media:* transverse section of stem, showing centripetal end of second cylinder; *c*, cortical cells; *x*, distinctly pitted xylem cells at tip of bundle; ×850.

There is no evidence of protophloem in connection with the cortical cylinder. A transverse section (fig. 11), which is thoroughly representative of the state of affairs, shows that practically all cells of the phloem are suberized bast fibers. This, together with the very apparent alignment of the fibers, is convincing proof that no protophloem is present.

Sections of the first cortical cylinder near the stem base reveal conditions almost identical with those of the normal cylinder, excepting that in the former both protoxylem and protophloem

[9] DORETY, HELEN A., The extrafascicular cambium of *Ceratozamia*. BOT. GAZ. 47:150–152. *pl.* 7. 1909.

are absent. Bundles of the former resemble those of the latter in shape, the secondary alignment of the former being disturbed by unequal growth and pressure, and bundles of both are of about equal size, those of the cortical cylinder being a little longer radially. Both cylinders give off leaf traces which differ in respect to the presence or absence of protoxylem and protophloem.

Other cortical cylinders.—There is little reason for believing that the second and succeeding cortical cylinders would have

Fig. 10.—*Cycas media:* radial section of stem, showing centripetal end of second cylinder; *c*, cortical cells; *ph*, crushed protophloem of first cylinder; *x*, innermost xylem elements of cylinder, showing distinct pits, some having fused; ×400.

a mode of origin and development different from that of the first; consequently but little time was devoted to the study of the second cortical cylinder. Preparations from the stem base only were examined, and, as was expected, these showed conditions in the mature part of the stem identical with those of the first cortical cylinder in the same region. Further discussion, therefore, would be but a repetition of what has been recorded thus far.

In concluding the matter of cortical cylinders it may be well to mention the relationship of their number to the age of the plant. Certainly they do not occupy the position of growth rings, nor

are they laid down at regular intervals of time. The plant which was studied was more than a century old and yet had but 3 vascular cylinders. Doubtless the cortical cylinders are related to certain activities of the plant alternating with long periods of rest which may vary greatly in point of duration.

FIG. 11.—*Cycas media:* transverse section of stem, showing tip of phloem belonging to second cylinder; *c*, cortical cells; *b*, suberized bast fibers; ×235.

Summary and conclusions

1. The paper deals with the adult stem of *Cycas media*, particular attention being paid to the xylem and phloem details of the normal and first cortical cylinders.

2. Not all the vascular cylinders are of equal longitudinal extent. Only the normal one begins its differentiation as high up as the meristem, the others beginning their differentiation successively lower, and each one in the cortex outside the next inner cylinder. The normal cylinder, therefore, is the only one which would be expected to originate with a procambium, and the only one which could develop protoxylem and protophloem.

3. Following up these expectations, both protoxylem and protophloem were found to have been developed during the early activities of the normal cylinder. Protoxylem elements are usually scalariform, although hints of spiral tracheids are more or less frequent. Primary xylem is scalariform and secondary xylem is characteristically pitted.

4. Neither protoxylem nor protophloem was found in the first cortical cylinder. Practically all of the xylem elements are pitted, but scalariform tracheids are occasional.

5. The secondary phloem of both cylinders is characterized by the great number of suberized bast fibers compared to the number of sieve tubes.

6. All cortical cylinders are similar in respect to their origin and development and are probably related in their appearance to the alternating periods of rest and activity of the plant.

7. Unfortunately material was unavailable which would have shown the beginning of differentiation of a cortical cambium. Such a piece taken from the stem apex would have been far enough up to destroy material needed for further research.

University of Chicago

ECOLOGY OF TILIA AMERICANA

I. COMPARATIVE STUDIES OF THE FOLIAR TRANSPIRING POWER

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 253

(WITH THIRTEEN FIGURES)

JAMES E. CRIBBS

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Introduction

Up to the present time transpiration studies have been conducted almost exclusively with potted plants, or upon plants which were growing under controlled conditions. This is due to the fact that most of the investigation has been purely physiological in its aims; and since the factors influencing this process are numerous, they can evidently be more definitely calculated in controlled habitats than in natural ones.

It has been the investigator's aim to carry this line of experimentation into the field, in an endeavor to determine what differences occur in the relative foliar transpiring power of a certain species when growing in different environments. It was at once recognized that the complication of factors which influence transpiration is considerably increased, and the precision with which they may be measured and the relative values to be attributed to each are perhaps less exact than when such investigation is made under greenhouse or laboratory conditions. Nevertheless, there are certain problems which of necessity must be worked out under field conditions, especially when we wish to determine the ecological value or relationship of the environment.

For this investigation *Tilia americana* was chosen because it, perhaps more than any other of our tree species, grows under a wide range of environmental conditions. This is especially true when we consider its unusual ability (as a member of a mesophytic forest) to surmount moving dunes which chance to advance upon it. This ability of *Tilia* to persist on the moving sands brings it

into a set of varying conditions which are widely different from those of its normal mesophytic environment, and affords an excellent opportunity to investigate certain ecological and physiological features which are brought under particular stress because of the abrupt change in growth conditions.

Five stations for investigation were selected on the dunes near Miller, Indiana, and will be designated here as *A*, *B*, *C*, *D*, and *E*.

FIG. 1.—Station *A*, showing *Tilia* on forested complex, associated with *Psedera*, *Smilacina*, *Acer*, and *Prunus*.

Studies were conducted also at station *F* in a mesophytic forest on morainic clays in western Pennsylvania, that a comparison might be had for different soil conditions.

Station *A* (fig. 1) is located on an established dune complex which has advanced to a state of mesophytism in regard to both the tree and herbaceous vegetation. It is well sheltered from the strong lake winds, and is not exposed to the intense light and the accompanying high temperatures of the open sand areas.

Station *A* lies deeper in the complex than *B* (fig. 2), which is situated at the base of a forested dune and is not more than 15 m.

from the edge of a blowout. The conditions are very similar at *A* and *B* so far as the humus is concerned. This has attained a thickness of approximately 5 cm., which is indicative of a relatively long period of stability. The presence of a well defined humus is correlated with a very rich development of herbaceous undergrowth and tree seedlings.

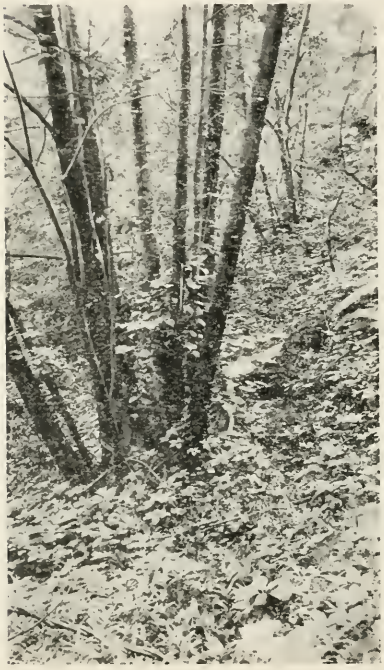

FIG. 2

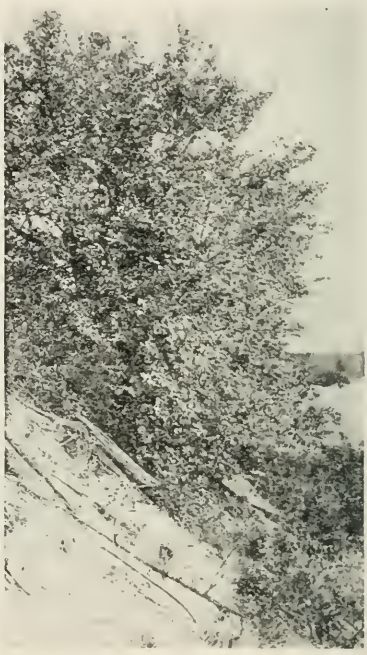

FIG. 3

FIGS. 2, 3.—Fig. 2, station *B*, showing position of *Tilia* at base of well established dune and with same plant associates; fig. 3, station *C*, where *Tilia* is located on edge of blowout; humus being broken up by sand movement, and access of wind and light much greater than at *A* or *B*.

Station *C* (fig. 3) is located on a west-facing slope on the edge of a blowout where there is a ready access to strong winds. The exposure to light is much greater here than at either *A* or *B*, especially in the afternoon when the sun's rays fall vertically upon the slope, while the stations in the forested complex are deeply shaded. The free sand movement has destroyed the humus and

with it a large part of the accompanying herbaceous vegetation, thus developing a situation of greater openness and more intense exposure.

Station *D* (fig. 4) is located on the lee slope of an advancing dune. In most respects this position is more xerophytic than that at *C*, for there is a complete absence of humus and herbaceous

Fig. 4

Fig. 5

Figs. 4, 5.—Fig. 4, station *D*, with *Tilia* on lee slope of advancing dune; sand advance rapid at this point, and absence of humus further inhibits herbaceous development; plant associates: *Cornus stolonifera*, *Ammophila arenaria*, and *Prunus virginiana;* fig. 5, station *E*, showing *Tilia* located on crest of high dune and exposed to most desiccating conditions found in dune environments.

undergrowth. The incipient rays are strongly reflected from the open sand, giving a very high light intensity and considerable ncrease in temperature. Being on a south-facing slope, the greatest exposure occurs in the late forenoon, followed early in the afternoon by shade which is continuous until evening.

Station *E* (fig. 5) represents the most exposed habitat to be found on the open sands, as it is situated about 25 m. above the

lake on the crest of an eroding dune which is exposed to wind from all points of the compass. The water content is less at this point than at lower levels and the light intensity becomes the greatest, the sun striking the station about 8:00 A.M., from which time it remains directly exposed until evening. The absence of humus and the exposure to wind combine to give a high sand mobility, which means a most unstable kind of habitat.

FIG. 6.—Station *F*, showing *Tilia* in mesophytic forest on clay soil; chief plant associates: *Osmorhiza*, *Adiantum*, *Caulophyllum*, *Aralia*, and *Actaea*.

Station *F* (fig. 6) is located in a forest, the chief tree members of which are *Acer*, *Liriodendron*, *Castanea*, and *Prunus serotina*. The herbaceous undergrowth includes such mesophytic species as *Aralia racemosa*, *Adiantum pedatum*, *Osmorhiza longistylis*, *Viola pubescens*, etc. The humus at this station has a depth of about 5 cm. and the underlying soil is a mixed morainic drift.

Methods

The cobalt chloride paper method was employed to determine the relative transpiring power. This method, first used by STAHL (15), has been improved and employed by subsequent investigators and is undoubtedly the one in present use which is best adapted for work in the field. Whatman's filter paper no. 30 was used throughout this work and was treated with a 3 per cent solution of cobalt chloride and prepared in accordance with the method described by LIVINGSTON and SHREVE (10). Preliminary tests were made with the plain paper and the tricolor slips of LIVINGSTON and SHREVE, and as essentially the same coefficients

were obtained the tricolor paper was discarded as being more difficult to prepare and less easily handled in the field. The plain paper has the additional advantage of being somewhat slower, and is thus more satisfactory when used at stations on the open sands, for the change in color was found to take place so quickly that even the paper with longer time values was frequently difficult to read. The probability of error arising from short time periods was largely eliminated by increasing the number of readings from the usual 5 to about 8 or 10, and the time recorded in each instance was the average of all readings made.

The paper was applied to the leaf surface by means of the clip devised and described by LIVINGSTON (8). At each of the stations leaves were chosen which were about 1 m. above the ground; readings were taken from the same relative position on the different leaves; and so far as possible the same set of leaves was employed in each subsequent day's work. Records were taken from 2 leaves at each station, usually at hourly intervals, beginning as soon as there was sufficient light to observe the color change and continuing until darkness prevented further reading. Records given here were taken from the abaxial (stomatal) side only.

The indirect method of determining the color change over the standard evaporating surface, as suggested by BAKKE (1), was used during the second summer's work, thus making it unnecessary to take the standard cup of LIVINGSTON and SHREVE into the field.

Various devices were tried for heating the hygrometric paper to force off the water of crystallization, for considerable difficulty was experienced because of the prevalence of stong lake winds, so a special lamp was devised and used for this purpose. The chief difficulty with the acetylene lamp was to get a steady flame for a long period of time. That which was finally employed consisted of a railroad lamp, with a round wick, which had an oil capacity of about 0.5 liter. A tubular piece of tin was fashioned so that it had a transverse dimension of about 8 cm. and a length of 23 cm. This was fitted to the base by means of 3 guides so as to leave a space of about 2 mm. below for ventilation. Into the

upper end was set a small tin cup such as may be obtained at any hardware store, and over this a screen lid was placed for use when working in a high wind. For ventilation above the flame, a ring of small holes was cut about 2.5 cm. below the level of the inserted cup. This lamp was found to be readily controlled, burns alcohol or kerosene equally well, can be used successfully in a strong wind, and burns continuously for 48 hours or more without refilling or adjustment.

Relative humidity was calculated hourly by means of the sling psychrometer, such as is in use by the United States Weather Bureau, and the wet bulb depression was referred to a standard table as given by MARVIN (12) to obtain the relative humidity values.

Soil temperatures were recorded for depths of 2 dm. and 4 dm. by means of a centigrade thermometer mounted on a cylindrical piece of hickory which was well adapted for inserting into the sand. Atmospheric temperature records were taken and recorded hourly during each day of experimentation.

Soil samples were collected at the different stations on the days when these were worked and the moisture computed on the basis of dry weight. From these results growth water was calculated by the equation GW = SW − WC, in which SW = total soil water and WC = the wilting coefficient. Samples were taken from depths of 2 and 3 dm. and dried for 6 days at a temperature of 100° C.

The wilting coefficient of the soil was computed by the centrifuge method of BRIGGS and MCLANE (4). The moisture equivalent was obtained directly and the wilting coefficient derived from this by the equation of BRIGGS and SHANTZ (3), $\frac{ME}{1.78} = WC$.

The evaporating power of the air was recorded by means of the porous clay cup of LIVINGSTON (7). Hourly readings were readily secured by mounting the atmometer on a graduated burette tube which was refilled when the water column fell to a point about 25 cm. below the level of the cup.

Field work was conducted under diverse weather conditions to discover to what extent the varying of such factors as relative

humidity, wind, light, temperature, etc., might influence the rate of transpiration in the field, and to compare its relative influence in the different environments. In each instance readings were taken at stations *A*, *B*, and *C* on the same day. The same may be said for stations *D* and *E*. Fig. 7 represents the records of relative transpiring power, temperature, relative humidity, and evaporation for *A*, *B*, and *C* on July 21, 1918. As in the following graphs, the scale to the left is for the index of foliar transpiring

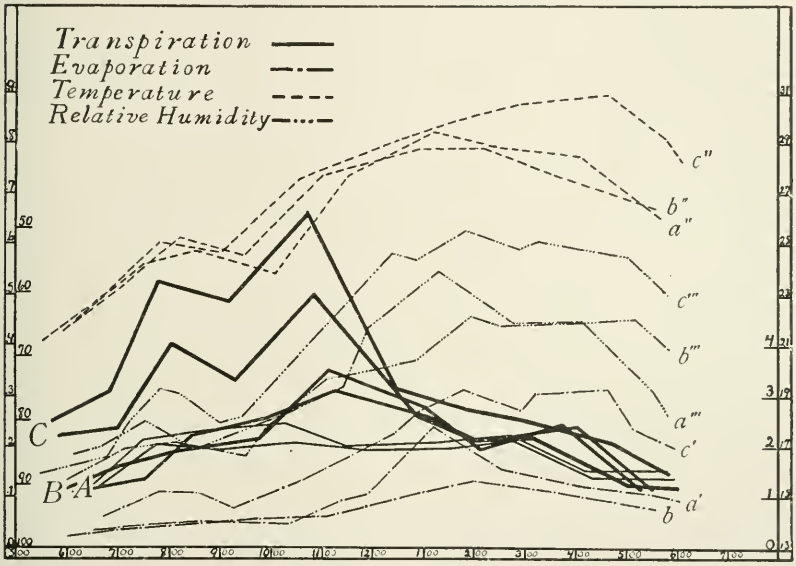

FIG. 7.—Graph giving transpiration, temperature, evaporation, and relative humidity curves of stations *A*, *B*, and *C* for July 21, 1918; scale similar in all following graphs (see text).

power; the inner one to the left is for relative humidity; the inner one to the right is for evaporating power of the air and is expressed in cubic centimeters per hour; and the scale to the right records the atmospheric temperature in degrees centigrade. Time is indicated at the bottom of each graph.

It will be noted that there is a close parallelism between the curves representing temperature, evaporation, and relative humidity. This is in fact what would be expected, as these factors are

closely interrelated. Accompanying the opening of the stomata in the morning, there is a rise in the transpiration indices, and their curves follow closely those of the factors just mentioned until about 11:00 A.M., when a marked divergence occurs. The transpiration curve declines rapidly from a maximum at this time, while the curves representing relative humidity, temperature, and evaporation continue to rise until about 2:00 P.M. following which there is a gradual decline. This feature is little in evidence in the graph of station *A*, but becomes more so in those of *B* and *C;* that is, the divergence becomes more and more conspicuous with an increase in exposure of habitat. This same feature will be found to recur with greater prominence when we consider the data of stations *D* and *E* on the open sand.

There has been considerable discussion concerning the interpretation of this sudden decline: LLOYD (11), following his investigation of stomatal action in *Fouquieria* and *Verbena*, concluded that the earlier view that stomatal movement controlled the transpiration rate was not entirely true, for there may be an increase in the transpirational loss for some time after the maximum opening; and there was found to be frequent decrease and subsequent increase in the afternoon, with little or no accompanying stomatal movement. RENNER (13) advanced the concept of "saturation deficit" to explain this behavior in transpiration, and LIVINGSTON and BROWN (9) in the following year discussed this behavior under the "incipient drying" theory. These are essentially the same concept, namely, that following the rise in the transpiration rate which accompanies the stomatal opening in the morning a point may be reached when the turgidity of the mesophyll cells of the leaf becomes sufficiently reduced to increase the surface tension of the water films in the walls. A check in the molecular diffusion of water from the cells follows. The increased concentration of the cell contents would then exert additional pull on the water in the translocating system, and a restoration of turgor may then follow without visible wilting occurring in the leaf. The length of time elapsing before the restoration of turgor depends largely upon the evaporating power and temperature of the atmosphere, also upon the relative humid-

ity of the air, and in some cases upon growth water. In recording the daily transpiration march in *Tilia* this saturation deficit was found to be very characteristic, especially at times when the relative humidity is low. It is also induced more readily when the growth water is near the zero point.

There is another condition, however, under which a very similar behavior in the graph is noted and may very easily be confused with the preceding. This would be especially liable to happen when one is employing the porometer and cobalt chloride paper only, as was done in the work of Trelease and Livingston (16). This cause lies in a sudden change in relative humidity. Thus if there is a sudden increase in relative humidity the effect on the transpiration index is to depress it. Such depressions may be detected from saturation deficits by an accompanying lowering in the curve of evaporation, for when it is due to a deficit the evaporation curve will be high. Inasmuch as a depression in transpiration caused by an increase in relative humidity is not accompanied by a closure of stomata, according to Lloyd (11), the porometer of course would continue to record the relative stomatal opening, and a divergence in the graphs would result. Instances of this appeared a number of times during the experiments, and were very marked on the occurrence of transient showers.

The sudden depression at 8:00 A.M. in graphs of station *C* (fig. 7) is due to this influence. It will be noticed that there is a corresponding increase in relative humidity, with a lowering of temperature and evaporation power. The behavior at this time is definitely attributed to the passing of a thundershower some distance to the south. It is interesting to note that the influence is more pronounced at station *C* than at *A* or *B*, especially as regards transpiration and evaporation. The drop in evaporation at *C* is attributed mostly to a sudden period of calm which had less effect at *A* and *B* because of their more sheltered position. The greater effect in the relative transpiration curve at *C* is probably to be interpreted as the result of a greater susceptibility of the leaf to a change in relative humidity when transpiring at a high rate.

The curves in fig. 8 were plotted from readings taken at station *D* on September 2. One of the outstanding features of this graph is the parallelism in all the curves. It will be observed that the maxima of transpiration coincide with those of temperature, evaporation, and relative humidity; and that there is an absence, or at least almost a complete absence, of an afternoon saturation deficit. In both of these respects this graph differs from the preceding one. The difference was found to be related to the atmospheric conditions. The evaporating power of the air

FIG. 8.—Graph plotted from data taken at station *D* on September 2, 1918; parallelism of curves and absence of saturation deficit due to high relative humidity.

was low throughout the day, never reaching 1 cc. per hour, while ordinarily at station *B* it became 2 cc. or more per hour by 2:00 P.M.; the temperature was low and the humidity high. This combination of factors in the field was always found to favor parallelism of curves and an absence of a saturation deficit. Under such conditions the maxima usually occur later in the day than when the deficit is developed.

At 7:30 A.M. there occurs a sudden fall in the transpiration indices which resembles the deficit drop. This was due to a sud-

den shower which began at 7:40 and lasted until 8:20. If the stomatal behavior is similar in *Tilia* to that found by LLOYD (11) to exist in *Fouquieria*, *Verbena*, and *Ampelopsis*, a porometer curve, if such had been made, would probably have continued to rise from 8:00 to 10:00 A.M. and would have given the type of curve that is obtained when a true deficit occurs. The evaporation curve alone shows that this was not a deficit depression, and one could safely infer the same from the much higher maximum that immediately follows; but if such a drop had occurred near midday, the second higher mode may not have occurred and a fall identical with that of the saturation deficit may have resulted. It will be noted that, notwithstanding the low evaporation rate, the transpiration index is quite high. This was found to be true for positions on the open sand, although under the same atmospheric conditions on humus the index was always much lower. This is probably largely due to the greater light intensity in the former position, and to a certain degree to higher temperatures.

It may be interesting to note the following atmospheric conditions in their general relation to the transpiration indices for this particular day: 5:00–7:20 A.M., partly cloudy; 7:20–7:40, cloudy; 7:40–8:20, rain; 8:20–9:30, cloudy; 9:30–10:00, clearing; 10:00–12:30 P.M., sun; 12:30–3:00 P.M., cloudy; 3:00–6:00 P.M., clear, but station shaded. From these data it will be seen that the drop from 1:00 to 3:00 P.M. was probably due to the sudden cloudiness of that period, which may have caused sufficient closing of the stomata to effect a depression. There is also a small drop in temperature and relative humidity at this time which would also have their influence, although not recorded by the atmometer. It should be said, however, that the white cup is less influenced by light changes than is the leaf (LIVINGSTON 6), and this was found to be most noticeable on the open sand when the index of transpiration was high.

One of the most striking relations that appeared in these comparative studies was found in the readings taken at the stations located on humus and those on the open sand during the latter part of the summer. As previously stated, stations *A* and *B* are in a forested complex which has a well developed humus

and an abundant herbaceous undergrowth, and *C* is on the edge of the same complex, where it is being destroyed by a blowout. These stations, when compared with *D* and *E*, stand out in contrast by their greater mesophytism. This is true in regard to the texture of their foliage and its richness; but during the months of August and September the *Tilia* complex rapidly undergoes a change which is very noticeable in the vegetation and is conspicuous in the relative transpiration indices. This change is initiated by an early reduction of the soil moisture to the wilting coefficient, evidently because of the heavy vegetation the sands are supporting and the excessive transpiration rates caused during this period by the highly desiccating atmospheric conditions. Stations *D* and *E* show, during this same period, a higher growth water content on the exposed dunes; and the abscission which is carried on rapidly in the *Tilia* complex is entirely unnoticed here until much later. The greater soil moisture on the open sand as compared with the pine and oak dune stages has been pointed out in the work of FULLER (5), and is seen to be similar for the *Tilia* complex. Fig. 9 emphasizes this relationship. The data for stations *A*, *B*, and *C* were taken on August 26, and that for station *E* on August 11. Although the 2 sets of readings were not taken at the same time, the atmospheric conditions on the 2 days were practically identical. Both days were sunny throughout, and the general parallelism of temperature, evaporation, and relative humidity was rather unusual. Curves for these factors were plotted for stations *A* and *E* only.

The transpiration indices of *A*, *B*, and *C* were all very low, that of *C* being slightly higher than *A* or *B*. They rose slowly in the morning from a low point and reached a low maximum at 8:00–8:30 A.M. A deficit occurred then because the soil moisture had reached the wilting coefficient, and the indices remained low throughout the day, rising slowly in the evening as the leaves regained their turgor. Visible wilting occurred about 10:30 A.M. at these stations, and was sufficient to cause stomatal closure. The temperature, evaporation, and relative humidity are seen to have remained high, reaching a maximum about 3:00 P.M., after which they declined rapidly.

A very different behavior occurred at station *E*, where the exposure is more intense. The growth water here was found to be 0.679 per cent at 2 dm. and 1.054 per cent at 3 dm., but the leaves remained turgid throughout the day, and there was no visible wilting. The transpiration index at 6:00 A.M. was quite high, and rose rapidly during the morning to a maximum at 12:00 noon. Here a saturation deficit was developed and a sudden

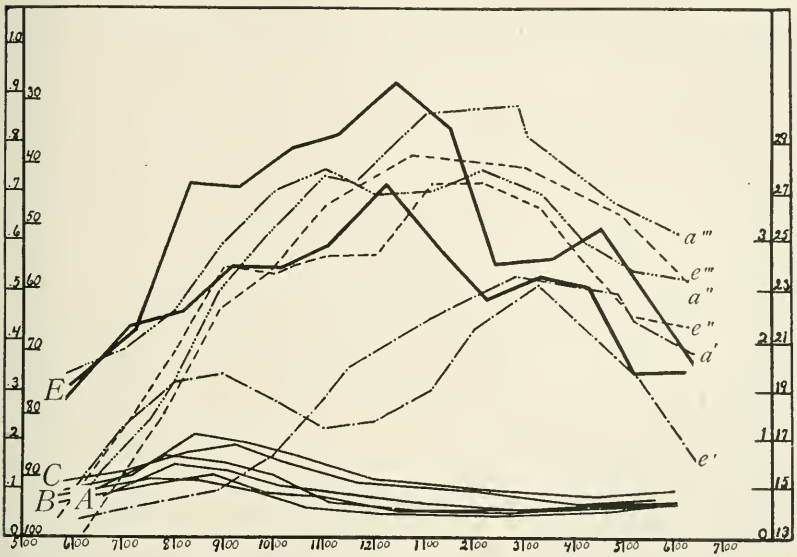

FIG. 9.—Graphs for stations *A*, *B*, and *C* on August 26 and station *E* on August 11, 1918; high transpiration index occurs on open sand with afternoon saturation deficit, while indices for humus stations remain low, due to visible wilting.

depression occurred until about 3:00 P.M., which was followed by a second low mode at 4:30.

This curve is a very typical one for a clear day at this position, in that there is a rapid rise in the morning to a high maximum, and a following clearly marked depression due to a saturation deficit, but no visible wilting occurs; then a second low mode in the afternoon about 4:00 P.M., followed by a rapid decline with the closing of the stomata in the evening. The morning maximum occurs from 9:00 to 12:00, unless disturbed in some way by

such influences as thundershowers and sudden shifting of wind to or from the lake with accompanying atmospheric changes in temperature, relative humidity, etc.

The usual pronounced saturation deficit that occurs on the exposed sands is shown in fig. 10, which is a graph of readings taken at station *E* on July 27. There was a very rapid rise to a maximum at 9:30 A.M., at which time the water loss presumably

FIG. 10.—Graph plotted for station *E* on July 27, showing typical saturation deficit developed in open dune environments on clear days with temperature high or relative humidity low.

equaled the maximum translocating ability of the plant under the conditions. Then as a deficiency was created in the cells by an excessive loss, a drop in the index occurred until the turgor was regained. A second low mode occurred at 4:00 P.M., a feature recurring in all records taken at stations *D* and *E*, in which an appreciable deficit was developed. Although the decline in the relative transpiration index was considerable in the early afternoon, there was no visible wilting.

Fig. 11 is a composite graph in which the curve for each station is plotted from the average of all the readings taken. This figure shows the relative transpiring power of *Tilia* in the different dune environments, and it will be seen that there is a very pronounced increase in the index of transpiration when considering the stations in their order from the mesophytic to the more xerophytic habitats.

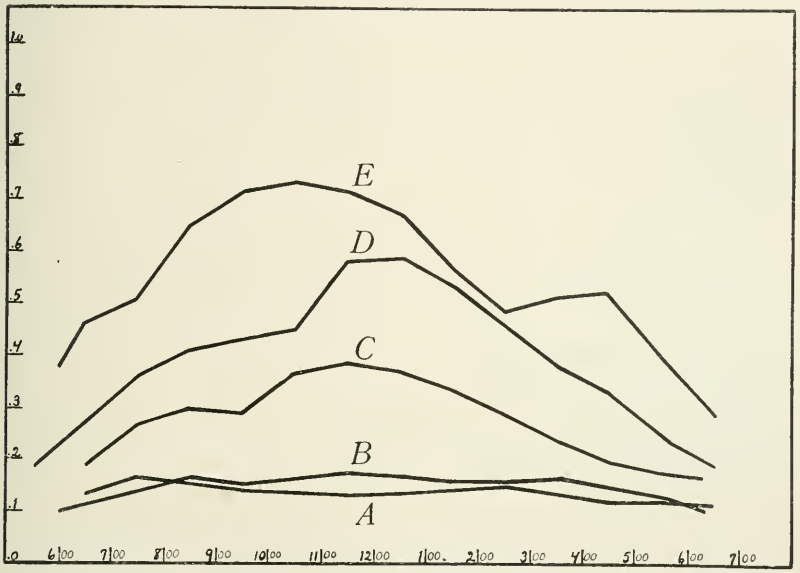

Fig. 11.—Composite graph of all transpiration readings taken at stations *A*, *B*, *C*, *D*, and *E*, showing increase in transpiring power accompanying increased exposure of habitat.

These curves being averages, and hence not recording daily variations, do not give to best advantage the typical daily curve. The occurrence of the maximum about 12:00 noon in the more mesophytic situations, however, and an earlier occurrence from 9:00 to 11:00 A.M. on the open sands, is noticeable even in the average graphs, as is also the earlier morning rise characteristic of the exposed stations.

Fig. 12 includes averages of all the different factors taken in connection with the dune transpiration studies. The unit spaces at the top of the graph have the following values: evaporation

5 cc. per unit; transpiration 0.1; relative humidity 15 per cent; soil temperature 7° C.; atmospheric temperature 10 per cent; and growth water 1 per cent per unit. From these it will be seen that the conditions existing at stations *A* and *B* are closely similar; but from *B* to *E* the stations represent a graded series of habitats as regards these factors, just as clearly as they do when we consider their comparative positions in the vegetative cycle. This graded variation found in the dune environments is much more pronounced than that for different situations on clays, which will

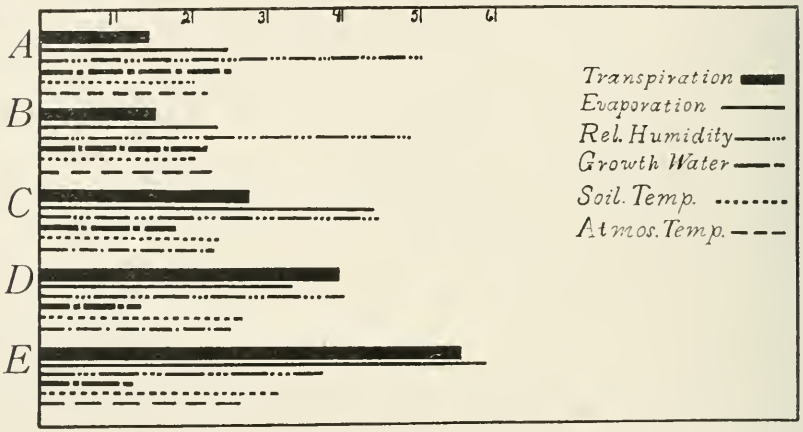

FIG. 12.—Comparative graph illustrating conditions of transpiration, etc., for the 5 dune environments; close gradation of factors evident here; increase in transpiration accompanying decrease in growth water is characteristic of dune habitats.

be mentioned only briefly in this paper. The increase in relative transpiring power that occurs with an increase in exposure of habitat is very evident.

Concerning evaporation, the only variation occurs at station *C*. Here the water loss is considerably more than at *D*. This is because of the greater access of wind at *C*, which is located on the edge of a blowout, while *D* is on a protected lee slope where only south and southeast winds have access.

The average relative humidity decreases rapidly from *A* to *E*, and is almost parallel with the increase in the transpiration index. Variation in humidity has been considered the most poten-

tial single factor influencing the transpiration index (Briggs and Shantz 2), and so far as considered in these studies it has been found to be a factor of the first order. It is not always the controlling one, however, for there are frequent variations in the daily march which owe their appearance to other causes. Temperature, stomatal movement, and growth water, for instance, at any given time may become the dominating factor, only to be succeeded as the conditions vary by some other which for the time being replaces it.

The proportional increase in the soil temperature over that of atmospheric temperature from *A* to *E* is of interest, and it may safely be inferred that high soil temperatures have a prominent part in the maintenance of the water supply, and hence indirectly concern the transpiration rate. Until more accurate quantitative measurements are made upon the effect of increase of temperature of the soil upon the water absorbing power of roots, however, the quantitative influence of this factor can only be speculated. Such work as has been done by Rysselberghe (14) on the effect of temperature changes on the rate of osmotic transfusion through semipermeable membranes leads to the conclusion that the much higher soil temperature, as of *E* over station *A*, must be one of the factors which enable the exposed plants to take over water from the soil with sufficient rapidity to withstand the much higher transpiration loss.

One of the noteworthy features of the dune studies appears in the relation of the growth water to transpiring power. It may be seen from the figure that there is a low average of growth water at all stations, that for *A* being only about 2.46 per cent and that for *E* 1.25 per cent. While it is true that the highest average is at *A*, the greater percentage here is due to the higher content during the earlier summer months. During the latter part of July and August, however, the water available for plant absorption decreases more rapidly than on the open sand, and the frequent drop in soil moisture to the wilting coefficient produces a period of stress and leads to early abscission. Meanwhile the growth water on the moving complex remains practically ncostant, especially at a depth of 3 dm. or below, where it is about

1.25 per cent or more. This greater constancy is attributed to the moving mulch of dry sand which breaks the continuity of surface films in the soil particles, thus preventing rapid evaporation. This ability of a plant to maintain a higher transpiration index with a growth water content of 1.25 per cent than the same species does with 20 per cent or higher, such as is commonly true for clays, indicates that the amount of growth water has but little relation to the transpiration index so long as the soil moisture content remains above the wilting coefficient. This gives a somewhat unusual aspect to the question of mesophytism and the part played by soil water as a factor in plant growth. So far as *Tilia* is concerned, it produces more vigorous vegetative structures, which retain their activities later in the summer, when growing in open situations on sand than when in the forested dune complex; and the factor of greatest importance seems to be, not the average growth water of the soil, but whether the available moisture repeatedly falls below the wilting coefficient. So long as it is above that point, although it is by only a very small percentage, the normal activities, including high transpiration, are carried on.

There is one point concerning this relation which needs investigation, however, namely, the relative extensiveness of the root systems in the two situations. I am inclined to the idea that the ability of *Tilia* to develop adventitious roots when covered by an advancing dune may enable such individuals to draw their water supply through a root system the absorptive surface of which is greater in proportion to the amount of foliage than is true of this species when growing in the forest complex. Another point of probable difference is that the individuals on the open sand may obtain a considerable portion of their water from a greater depth than do those on the humus; but the extent to which the root systems persist when submerged by advancing dunes has never been worked out.

As indicated by the averages shown in fig. 11, the situation at *E* is distinctly more xerophytic than at *A*. Every fact recorded in the experimentation points to this conclusion. The size,

general shape, and texture of the leaves at station *E* are also distinctly xerophytic, but it has been noted that notwithstanding this the transpiration index is higher, a fact not at all in accordance with the behavior of desert plants so far reported, for they are characterized by a low transpiration index, as pointed out by several workers. With this fact in view BAKKE (1) has suggested the foliar index of relative transpiring power as a test for the mesophytism of a plant, as follows: "As a result of the preliminary study, it may be suggested that plants exhibiting a diurnal foliar transpiring power of less than 0.30 may be regarded as xerophytes, while those exhibiting indices above 0.70 may be considered mesophytes." It may be seen at once that the behavior in *Tilia* is the reverse of that common to desert plants, and hence an application of this test would lead to confusion. I believe with BAKKE, however, that with certain reserve this method may be used as a fair indicator of mesophytism, provided two precautionary measures are taken: first, that the species under consideration be chosen in its normal environment and not in an abnormal or forced one; and second, that hourly readings be taken for at least two full days and that the relative humidity and temperature conditions be carefully employed in calculating the results. This latter precaution is necessary because of the great variation in the index at different times of the day, in the first place, and because of the wide variation of the indices at any particular hour on two successive days when the relative humidity or temperature has undergone considerable change.

The development of a xerophytic leaf under unusual conditions of exposure has been found in *Tilia* to result in a leaf less effective in preventing water loss than are desert types. This may be attributed to a lagging of the effect behind the causal factors. On the other hand, such lagging may be considered as not occurring, for there is always a favorable balance in water relations which permits a greater vegetative activity than would be possible in desert plants, and becomes possible on the open sands only because of a sufficient and permanent growth water throughout the growing season.

At station *F* there is considerable difference in the environment, and the factors accompanying it, from that of the dune series. It has already been noted from the introductory description that the undergrowth is composed of a more shade-requiring assemblage than even the most mesophytic positions found on the established dune complex. The humus is slightly more developed, but the soil underlying it is very different, and unlike in the forested dune habitats the growth water was always adequate to support an abundant and diversified vegetation, and never reached the wilting coefficient. The average available water at 2 dm. was 19.40 per cent and the minimum 12.45 per cent. Thus if growth water is inducive of a high transpiration, one would expect to find it here, but the average transpiration at this station for 5 complete days' readings was only 0.16, an index practically identical with that of station *A* of the *Tilia* forested complex where the growth water averaged 2.5 per cent.

The same tendency displayed by the dune graphs to show curves with a single mode recurs here where the mesophytism is greater. The maximum power of transpiration comes later in the day, commonly from 12:00 to 2:00 P.M., and is more frequently coincident with the maxima of temperature, evaporation, and relative humidity. This relation is shown in fig. 13, which is a graph of station *F* on June 14. At this time there was a growth water content of 26.74 per cent and a relatively low humidity. The temperature was low, while the evaporation was high when compared with the transpiration, higher than commonly found in dune environments. The morning rise of the transpiration index was very slow and the maximum reached was not very high. On this particular day it was clear until about 2:00 P.M. with increasing cloudiness through the afternoon, followed by showers at 8:00 P.M. Although it was clear during the forenoon the station was shaded throughout the period.

The fact that direct sun upon the leaves in a habitat of this sort almost always leads to a rapid increase in the transpiration rate would suggest that the low light intensity of the densely shaded forest is one of the chief factors leading to the low average commonly found there.

Summary

1. Cobalt chloride standardized paper was found well suited for comparative studies in the relative transpiring power of leaves in the field.

2. The daily march of transpiration in *Tilia* was found to vary greatly for the same leaf on different days. This variation was found to be influenced by relative humidity, temperature, light intensity, soil moisture, and presumably by soil temperature.

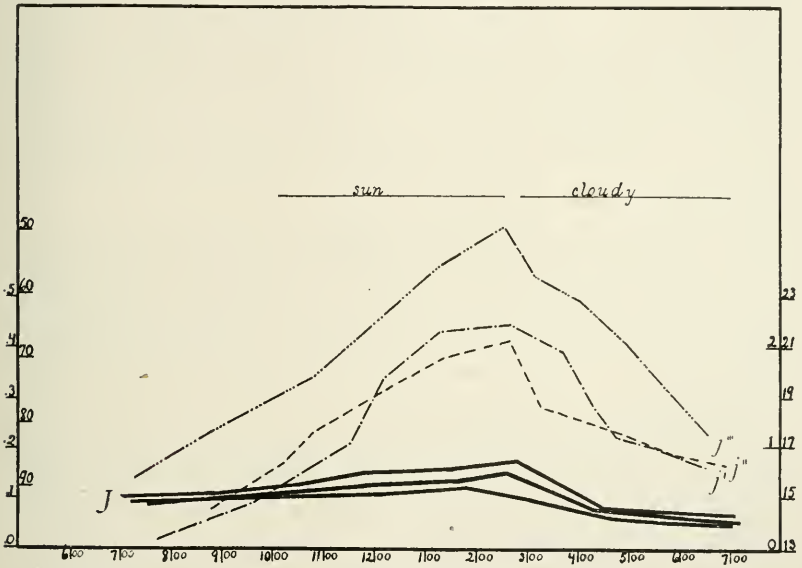

FIG. 13.—Typical graph illustrating low transpiration indices occurring in morainic mesophytic forest where growth water is always high; note low transpiration for a day with so low a relative humidity.

3. The foliar transpiring power of *Tilia* was found to increase in dune environments from an index of 0.15 on the forested *Tilia* complex to 0.55 in the most exposed situations on the open sand. In habitats between these extremes the transpiration power was found to be directly proportional to the relative exposure.

4. The morning rise in the daily march is more rapid on the open sand, where it reaches a maximum 1 to 2 hours earlier than in forested situations.

5. In the forested complex the curve representing relative transpiration tends to develop a single mode about midday, and this maximum tends to coincide with the maxima of temperature, relative humidity, and evaporating power of the air. The greater the mesophytism and relative humidity the more striking becomes this tendency.

6. In the most exposed situations on the open sand the relative transpiration maximum usually appears about 10:00 A.M., while the maximum temperature, relative humidity, and evaporating power occur from 2:00 to 4:00 P.M. This divergence from parallelism is due to the development of a saturation deficit, which appears successively earlier as the exposure of the habitat increases. The more mesophytic the habitat the less noticeable becomes this deficit, until it disappears entirely, especially on humid days.

7. The foliar transpiration index is influenced less by wind currents than is the porous cup atmometer.

8. Transpiration curves showing a saturation deficit depression usually develop a second mode about 4:00 P.M., which is, so far as noticed, always lower than the mode preceding the deficit depression.

9. Bimodal transpiration curves have been found to be due either to a saturation deficit or to a sudden increase in relative humidity, although lesser depressions may result from fluctuating temperature or intensity of light.

10. No evidence of visible wilting occurred in *Tilia* on the open sand at any time during the summer, although the so-called "incipient drying" was a common feature of the stations throughout this period. On the forested complex, however, visible wilting occurred during the first week in August because the vegetation was so dense that the water content of the soil was reduced to the wilting coefficient quite early.

11. The average mesophytism on the *Tilia* complex is considerably greater than on the open sand, the growth water averages being 2.5 and 1.25 per cent respectively; but the open positions are practically constant in their water relations, while the forested complex represents a decreasing mesophytism as the summer

advances, the growth water in the spring being higher than at any other time.

12. The amount of growth water in the soil apparently has very little influence on the transpiration index, unless it is reduced to the wilting coefficient. It might be argued that a low growth water is the cause of the saturation deficit depression, but there is evidence that it is due rather to the inability of the translocating system to conduct water to the leaves with sufficient rapidity to offset the transpiration loss, and not to a slowing up of the absorption rate. This is substantiated by the occurrence of the typical deficit in readings on *Tilia* when the growth water was greater than 20 per cent. The drop that occurs when the soil moisture falls to the wilting coefficient is more permanent and is due to stomatal movement which accompanies visible wilting.

I wish to express my grateful appreciation of the encouragement and suggestions given by Dr. GEO. D. FULLER, of the University of Chicago.

COLLEGE OF EMPORIA
EMPORIA, KAN.

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NOVEMBER 1919

CHEMICAL CONSTITUENTS OF AMARANTHUS RETROFLEXUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 254

M. L. Woo

(WITH ELEVEN FIGURES)

Introduction

It is a well known fact that weeds retard the development of cultural plants. This is due to a number of causes: use of water, shading, use of nutrient salts, etc. It has been claimed for various species of *Amaranthus* that they not only absorb nitrates to care for their nutrient needs, but that they store much nitrogen as nitrate. If this be true, this genus has an excellent adaptation to enable it to combat cultural plants, for nitrate supply is a common limiting factor for crop growth. In order to investigate this statement, to locate the place of nitrate storage, and to determine the amount of nitrogen used otherwise by this plant, separate analyses were made of roots, stems, leaves, and branches of *Amaranthus retroflexus* at various stages and under various conditions of growth. The amount of the several carbohydrates was also determined in each analysis, in order to calculate the carbohydrate-nitrogen ratio which is lately receiving so much attention. A tissue analysis of the seeds was also made in an endeavor to ascertain more fully the chemical constituency of this plant, with the hope of learning more of the peculiar germinative behavior of these seeds.

Historical

The first chemical analysis of *Amaranthus* was made by BOUTIN (1), a French chemist, in 1873. Housewives of that time were using these plants to clean their cooking utensils. This fact gave it some commercial importance and brought it to the attention of chemists. It was thought that the ability to "cut grease" must be due to acids in the plant. To verify this BOUTIN incinerated 100 gm. dry weight of the entire plant, and obtained 16 gm. residue. Water was added to leach out the soluble salts. The soluble portion weighed 8 gm. He called this potassium carbonate, and calculated the equivalent weight in grams of potassium nitrate, and found it to be 11.68, or 11.68 per cent. BOUTIN concluded that this plant was neutral in reaction on account of the presence of the neutral salt KNO_3 (as a matter of fact this plant is acid in reaction). Later (2) he made analyses of the other species of *Amaranthus* by the same method, to determine the amount of KNO_3. The result of his analysis was as follows: *A. atropurpureus* contains 22.77 per cent KNO_3 (one kilogram gives 31 gm. N and 103.5 gm. K); *A. Blitum* contains 11.68 per cent KNO_3; *A. ruber* contains 16 per cent KNO_3 (one kilogram gives 22 gm. N and 72 gm. K). It is evident that BOUTIN'S method is not an accurate quantitative method of determining the amount of nitrate present. On the other hand, he demonstrated by other qualitative methods that the several species of *Amaranthus* studied contain a large amount of potassium nitrate. BROSSET (3) suggests the use of these plants as a fertilizer.

PAMMEL and DOX (15), in 1917, made microchemical tests of three common pigweeds, *A. blitoides*, *A. graecizans*, and *A. retroflexus*, and found them to contain abundant starch, some protein, and a little fat. In addition they made the Kjeldahl-Gunning nitrogen determination and found these species to have 1.88, 2.32, and 2.49 per cent of nitrogen. Multiplying by the factor 6.25, they obtained 11.75, 14.52, and 15.59 per cent of protein respectively.

HARDING and EGGE (8) made an analysis of the seeds of *A. retroflexus* for fats, protein, starch, sugars, hemicellulose, crude fiber, and tannin.

Of late much significance is being attached to the carbohydrate-nitrogen ratio of plant tissues, or, as FISCHER (6) puts it, the $\frac{C}{N}$. On the basis of work done by him and others, FISCHER makes the following generalizations. If the value of $\frac{C}{N}$ rises by an increase in the amount of carbon, or by a decrease in the amount of nitrogen furnished the plant, there is an increase in the amount of flowering. If the value of $\frac{C}{N}$ drops by a decrease in the amount of carbon, or by an increase in the amount of nitrogen furnished the plant, there is an increase of vegetative growth and a reduction of flowering. Briefly stated, a great preponderance of carbohydrates in plants favors flowering. Since the carbon of plants is fixed from the carbon dioxide of the air by photosynthesis, conditions that favor photosynthesis will tend to increase the ratio, and according to FISCHER the flower production. He found that increased partial pressures of carbon dioxide in the air had this effect. Since nitrogen is absorbed from the soil in the form of nitrates, conditions that favor nitrate absorption will decrease the ratio, and according to FISCHER favor vegetation.

KRAUS and KRAYBILL (12), on the basis of much more critical work, including numerous cultures, tissue analyses, and microchemical and anatomical studies, conclude that a very high $\frac{\text{carbohydrate}}{N}$ value gives little vegetation and little or no reproduction; a medium $\frac{\text{carbohydrate}}{N}$ value gives moderate vegetation and good reproduction; and a low $\frac{\text{carbohydrate}}{N}$ value gives vigorous vegetation and little reproduction. Through their extreme conditions of culture, withholding nitrates, it is probable that KRAUS and KRAYBILL got much higher carbohydrate plants than FISCHER obtained in his cultures, hence their conclusion that very high $\frac{\text{carbohydrate}}{N}$ gives little vegetation or reproduction. In short, FISCHER worked only on the portion of the $\frac{C}{N}$ curve that induced fair vegetation and good reproduction or extreme vegetation and little reproduction, but not on the extreme of the curve that greatly

reduces both vegetation and reproduction. KRAUS and KRAYBILL cite literature showing that various conditions that greatly retard growth produce high carbohydrate plants. It seems that such conditions retard the use of carbohydrates for building new tissue to a greater degree than they do photosynthesis, and thereby lead to an accumulation of carbohydrates.

HEDLUND (9) finds that under like cultural conditions those varieties of winter wheat that have a higher percentage dry weight in the autumn are generally more winter hardy than the ones having a low percentage dry weight, and that cultural conditions that make for high percentage dry weight in any variety also make for winter hardiness. He finds, as do KRAUS and KRAYBILL, that high percentage dry weight is due to high percentage carbohydrate, and therefore high $\frac{\text{carbohydrate}}{\text{N}}$.

RIBERA (16) finds that all cultural conditions that increase the percentage dry weight in wheat decrease lodging. From this and the two investigations previously mentioned it is evident that high $\frac{\text{carbohydrate}}{\text{N}}$ increases straw strength and decreases lodging. High percentage of carbohydrate is said to increase hardiness, at least in part, by the greater amount of glucose present, and it may increase straw strength by inducing greater development of mechanical tissue along with greater thickness of walls, as KRAUS and KRAYBILL found for certain tissues of the tomato.

Methods and results

GREEN PLANT AT VARIOUS STAGES OF DEVELOPMENT

Preparation of samples.—Samples were secured on June 3, June 20, and July 8 consecutively from a vacant lot on 59th Street and Ingleside Avenue, Chicago. On June 20 samples were taken from two places, namely, the manure pile (rich soil) and the knoll (poor soil) for comparative work. The soil particles adhering to the roots and rootlets were removed by running water from a filter pump. As the velocity of water was very great, the soil particles were removed without difficulty. The roots were partially dried by the air current from the laboratory air line, and

finally dried by the use of paper towels. The roots, stems, and leaves were detached for separate analysis. The roots and the main stems were separated by cutting just between the cotyledon scar and the first branching rootlet. The leaf blades with the petioles were separated from the stem. Each portion was weighed and the length and diameter measured. Table I gives a brief description of the three consecutive samples.

TABLE I

THE GREEN PLANT AT VARIOUS STAGES OF GROWTH

Measurement	June 3 collection	June 20 collection	July 8 collection
	Inches	Inches	Inches
Average height	1.00–4.00	6.00–8.00	20.00
Taproot length	1.00–1.50	4.00–6.00	4.00– 6.00
Taproot diameter		0.10–1.00	1.00– 1.20
Secondary rootlets length	None	0.10–1.00	8.00–14.00
Secondary rootlets diameter	None		0.13– 0.25
Stems length	1.00–4.00	6.00–8.00	20.00
Stems diameter	0.13–0.25	0.37–0.75	0.13– 0.25
Lateral branches length	None	0.10–1.50	14.00
Lateral branches diameter	None		0.25– 0.50
Seed head	None	None	0.25– 1.00
Green weight	Grams	Grams	Grams
Roots	26.00	28.50	29.20
Main stems	52.05	97.40	88.30
Branches	None	None	80.45
Leaves	188.90	142.20	110.25

The green samples were then immediately put in a freezing chamber, allowed to freeze overnight, and ground in a meat grinder the next morning. The freezing prevents losses. In the frozen condition no juices ooze out or spatter in the manipulation. The samples were boiled with 95 per cent alcohol to destroy the enzymes, and were then transferred to extraction cups, with filter paper thimbles, previously dried and weighed. The tissues were fractionated according to KOCH'S (11) scheme for tissue analysis, namely, the lipin or ether soluble fraction (F_1), the alcohol water soluble fraction (F_2), and the insoluble fraction (F_3). In the green plant F_2 was comparatively small, consisting of chlorophyll and extracts of various pigments. The F_1 was put together with F_2 for the following carbohydrate and nitrogen estimation.

ANALYSES

Nitrogen compounds

NITRATES.—The nitrates were determined by the Schlösing-Wagner method (14) as modified by KOCH for use in his laboratory. The modification consists essentially in the use of an inverted burette instead of a tube sealed at one end, and of the Van Slyke apparatus (only volumetric tube and Hemple pipette), to measure the true volume of nitric oxide. The principle of the method is: $3Fe^{++}+NO_3^-+4H^+ \rightarrow 3Fe^{+++}+NO\,gas+2H_2O$. Therefore 1 mol. NO gas gives an equivalent of 62 gm. of NO_3, or 1 cc. of gas $= \frac{62}{22400} = 2.77$ mg. NO_3.

In order to determine the accuracy of this method, a known solution of KNO_3 (0.5 per cent) was used. Four consecutive determinations with 10 cc. of the known solution were made. The average volume of nitric oxide gas for each 10 cc. solution, calculated to standard condition, was 11.12 cc. The theoretical volume for 10 cc. of 0.5 per cent KNO_3 is 11.078 cc.

The determination of nitrates in the samples was made by taking an aliquot of the soluble fractions (F_1 and F_2). The nitric oxide gas driven over was caught in an inverted burette which had previously been filled with 40 per cent NaOH to absorb the CO_2 and neutralize the hydrochloric acid (HCl gas will come over when the hydrochloric acid concentration reaches 20 per cent in the boiling flask). The burette containing the nitric oxide gas was set aside and allowed to cool to room temperature; then the nitric oxide gas was transferred to the Van Slyke apparatus. The total volume and the volume of unabsorbed gas were recorded. The absorbed volume by the alkaline $KMnO_4$ in Hemple pipette is that of nitric oxide. This volume was then calculated to standard volume from temperature and barometric pressure, for example:

	I	II
Aliquot in cc. used	25.0	25.0
Total volume of gas (nitric oxide+air)	4.80	4.85
Volume of unabsorbed gas (air)	1.27	1.26
Volume of (absorbed) nitric oxide	3.53	3.59
Barometric pressure = 746.9; temperature 20.5° C.		
Volume at standard condition	3.23	3.27

	I	II
Equivalent in milligrams of NO_3	8.95	9.06
Milligrams of dry substance (25 cc.) used	107.75	107.75
Percentage NO_3 in soluble fractions F_1+F_2	8.30	8.41
The percentage soluble fractions in whole samples	45.9	45.9
Therefore percentage of NO_3 calculated on whole sample	3.81	3.86

Soil samples were taken at the same time that the green plants were gathered. The nitrates were estimated by the colorimetric method with phenoldisulphonic acid. The moisture in percentage and nitrates in parts per million are shown in fig. 1.

On June 20 the samples from the manure pile and from the knoll were taken for comparison. The nitrate content of the soil and that in the plant were as follows:

	Knoll sample	Manure pile sample
NO_3 in ppm in soil	300	29
NO_3 in percentage in plant (stem only)	1.71	1.45

The high NO_3 content in the soil of the knoll sample is probably due to the fact that some one, perhaps the gardener, had disturbed the soil by dragging his cultivator over it accidentally in cultivating his plot near by. The second reason is the better drainage and aëration in the knoll, and therefore better conditions for nitrification; but the striking fact is that high nitrate content in the soil did not bring about a proportional high nitrate content in the green plant organs. The rate of absorption increases with the aging of the plant; when the plants were about 25 days old, the nitrate in the stem was only 1.71 per cent. Eighteen days later (July 8) the nitrate content had risen to 8.58 per cent. During the same period branches grew from 0.1 to 14 inches, and their nitrate content rose to 12.50 per cent. This rapid increase in absorption of nitrates may partially be explained by the increase in extent of the absorbing roots from a radius of a few inches to about 2 ft.

The nitrate content in the roots, stems, and leaves is given in table II and is also shown in fig. 1. The nitrate content of the roots falls gradually from 1.85 per cent on June 3 to zero on June 20. At the same time nitrates in the leaves fall from 1.38 per cent to zero, while in the stem there is a gradual increase. There must be a definite reason for such differences. The differences may be due

to many causes. In the leaves protein synthesis is going on continuously in the presence of soluble carbohydrates. There is also synthesis of other organic nitrogen compounds, such as chlorophyll,

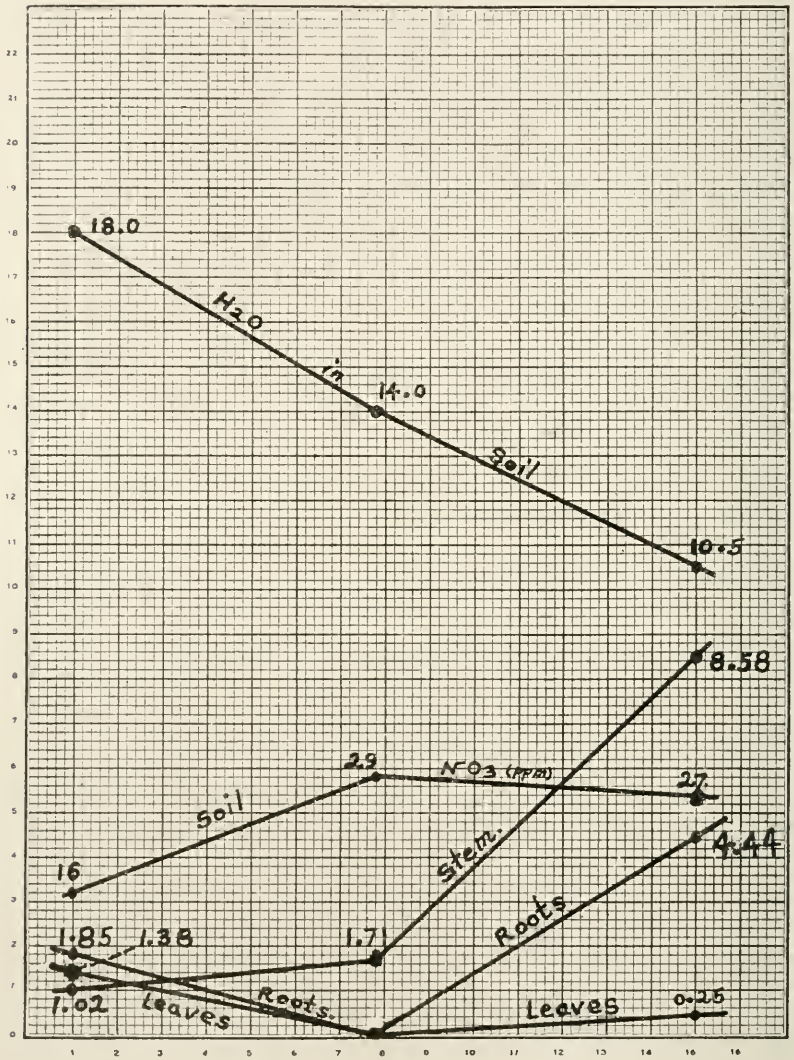

FIG. 1.—Relation of soil nitrification and nitrate intake by green plant organs; nitrate in soil expressed in parts per million; all other data calculated as percentage on dry weight basis.

phospholipins, etc., and all of the nitrates of the leaves seem to be used up in these syntheses. The nitrates are carried from the roots to other parts of the plants as fast as they are taken up from the soil. There may be as high a concentration of nitrates in the roots as in the soil (29 parts per million). This low concentration cannot be estimated by this method and would therefore be missed.

The stem and branches are the primary nitrate storage organs. The nitrate content rises as high as 8.58 per cent in the stem and 12.5 per cent in the branches during the early seed formation period. This high content is shown still more clearly by the ratio of nitrate nitrogen to the total nitrogen. This is 32.8 per cent for the roots, 51.85 per cent for the stems, 56.4 per cent for the branches, but only 1.25 per cent in the leaves. Curves showing this ratio in these organs at different stages are given in fig. 2. This large supply of nitrates in the stem and branches may be drawn upon heavily for further growth and seed production, although the supply seems more than adequate for these uses. There is also no reason for thinking that nitrate absorption ceases at this time. The extent to which this storage of nitrate is drawn upon by later development could be ascertained by the analysis of a set of samples taken late in the fall when seed formation and growth were complete. It is to be regretted that circumstances made such an analysis impossible for this paper.

It is worthy of note that the nitrate storage organs are the ones that made the most rapid growth in length, weight, and volume. The stem which rose from 8 inches on June 20 to 20 inches on July 8 at the same time increased in nitrate content from 1.71 to 8.58 per cent on dry weight basis. In addition to the stem there are numerous side branches which elongated from 0.1 to 14 inches in 18 days, making nearly 1 inch in 24 hours. At the same time there was an increase in percentage of nitrates per gram of dry matter from 1.71 on June 20 to 12.50 per cent on July 8. The rate of nitrate intake per gram per hour seems to follow a geometrical progression in each individual plant.

It appears that *Amaranthus* may be a very considerable factor in depleting soils of their nitrates. Also in case the weeds are burned the nitrogen stored is permanently lost from the soil. It

is a point of interest to know how generally this great power of absorbing and storing nitrates is possessed by weeds.

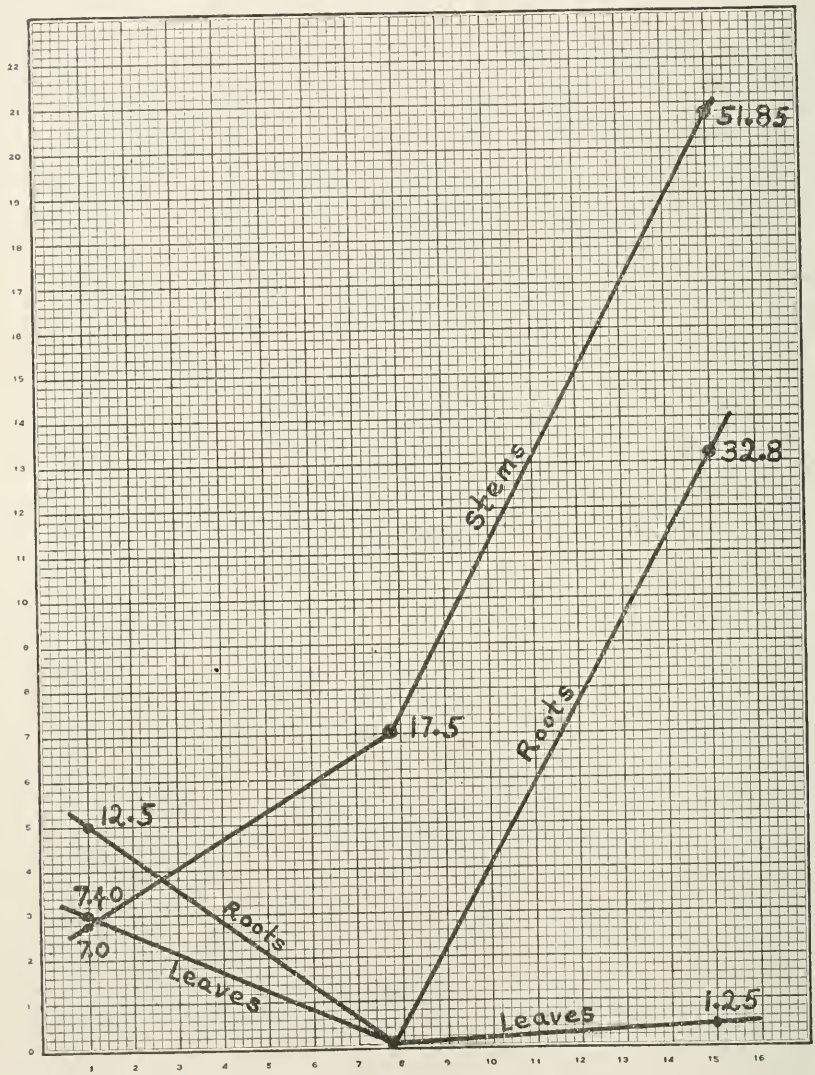

FIG. 2.—Ratio of nitrate nitrogen to total nitrogen; ratio for branches (not shown in figure) 56.4.

AMINO N.—The amino nitrogen was determined by the Van Slyke apparatus. The amino acids thus determined are chiefly

of the mono-amino-monocarboxylic acids. In each estimation only 2 cc. of the solution was used. The amino acid nitrogen deter-

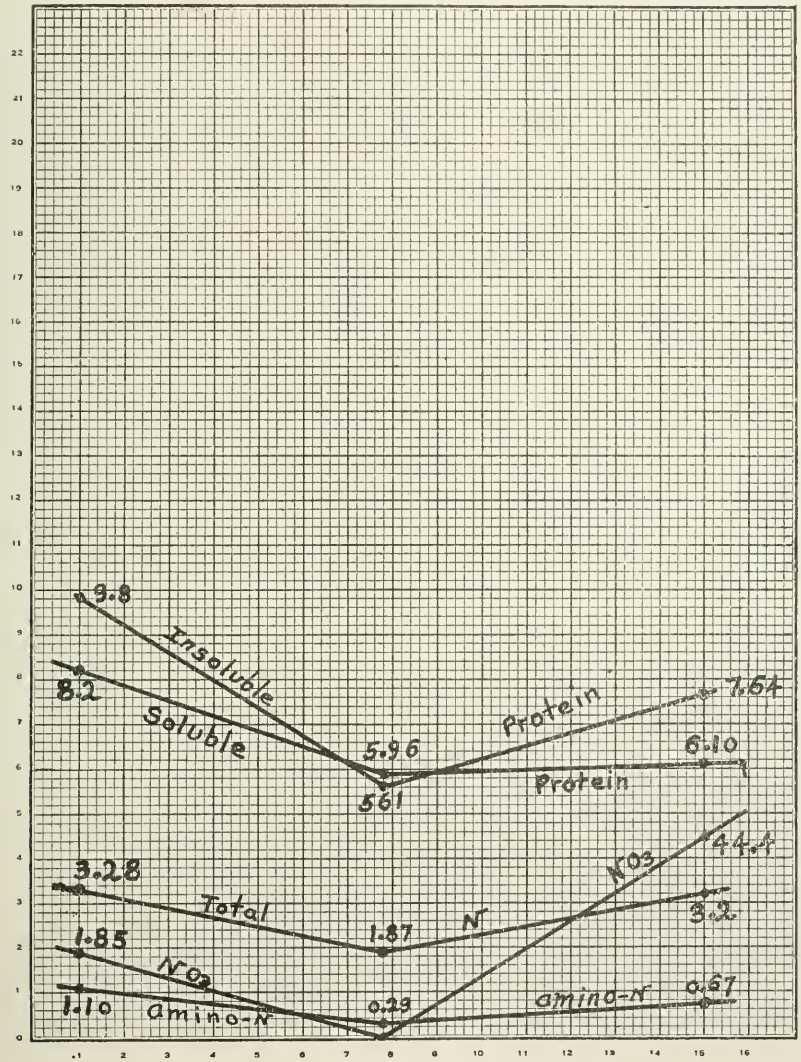

FIG. 3.—Nitrogen compounds in roots

mined throughout the season is given in table II. Curves showing the variation in roots, stems, and leaves are given in figs. 3, 4, and 5 respectively. In all the three organs the variation is very small.

In general the amino N varies directly with the insoluble protein; with a high protein content, high amino N; and low protein content, low amino N.

TABLE II

THE NITROGEN COMPOUNDS IN THE GREEN PLANT

Material	Roots		Stems		Branches		Leaves	
	June 3 collection (1–4 inches)							
Total N	3.33	3.33	3.19	3.21	....	....	4.25	4.27
Nitrates NO_3	1.77	1.93	0.99	1.04	....	....	1.36	1.40
Nitrate N	0.40	0.43	0.22	0.23	....	....	0.31	0.32
Amino N	1.09	1.13	0.43	0.43	....	....	0.97	0.96
Insoluble N	1.62	1.52	1.74	1.73	....	....	3.21	3.23
Insoluble protein	10.18	10.25	10.92	10.80	....	....	20.20	20.30
Soluble protein	8.24	8.17	7.67	7.92	....	....	4.58	4.52
	June 20 collection (6–8 inches)							
Total N	1.85	1.88	2.39	2.21	....	....	4.56	4.51
Nitrates NO_3	None	None	1.67	1.75	....	....	None	None
Nitrate N	None	None	0.38	0.40	....	....	None	None
Amino N	0.29	0.29	0.19	0.19	....	....	1.38	1.37
Insoluble N	0.86	0.91	0.95	0.97	....	....	3.66	3.59
Insoluble protein	5.40	5.72	5.86	6.10	....	....	23.00	22.6
Soluble protein	5.92	6.10	5.28	5.41	....	....	5.66	5.78
	July 8 collection (20 inches)							
Total N	3.21	3.12	3.74	3.80	5.04	4.94	4.85	4.80
Nitrates NO_3	4.41	4.47	8.40	8.75	12.50	12.40	0.25	0.246
Nitrate N	0.99	1.02	1.90	1.98	2.85	2.80	0.06	0.06
Amino N	0.63	0.70	0.35	0.34	0.48	0.49	1.44	1.42
Insoluble N	1.29	1.14	0.95	0.91	1.01	0.98	3.29	3.21
Insoluble protein	8.10	7.17	5.96	5.71	6.35	6.15	20.62	20.08
Soluble protein	6.16	6.05	5.60	5.70	7.42	7.30	9.42	9.62

INSOLUBLE PROTEIN.—The insoluble protein was calculated from nitrogen of the insoluble fraction (F_3). The insoluble protein is given in table II, and curves showing fluctuation during the growing season are given in figs. 3–5. The insoluble protein falls and then rises again at maturity in the root, while in the leaves the fluctuation is in the opposite direction (see curves). In the stem the decline is in the early stage from 10.89 per cent (June 3) to 6.03 per cent (June 20). From that time on the curve is almost a straight horizontal line; therefore the rate of synthesis of the insoluble protein must have been keeping pace with the growth of the stem,

because the fall at this time is only from 6.03 to 5.84 per cent (almost within experimental error).

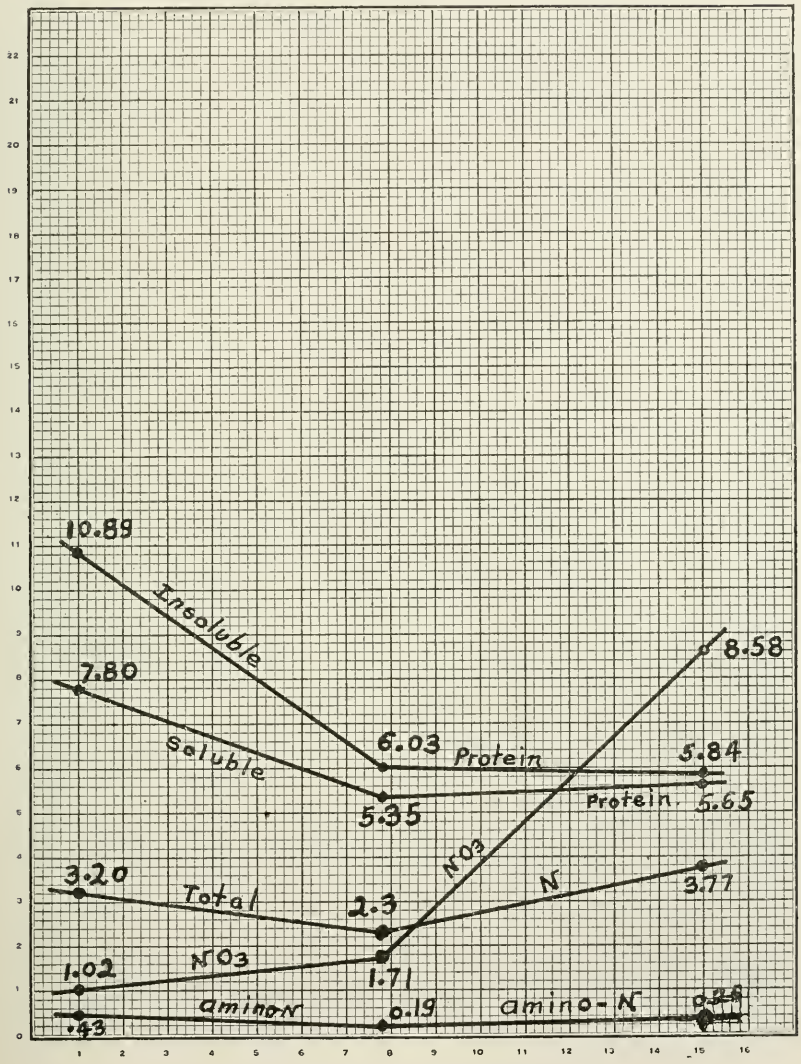

FIG. 4.—Nitrogen compounds in stems

SOLUBLE PROTEIN.—Soluble protein in F_1 and F_2 is computed from the Kjeldahl nitrogen determination. The nitrate nitrogen

was determined separately (by the method previously described); so the Kjeldahl determination was conducted without any modifica-

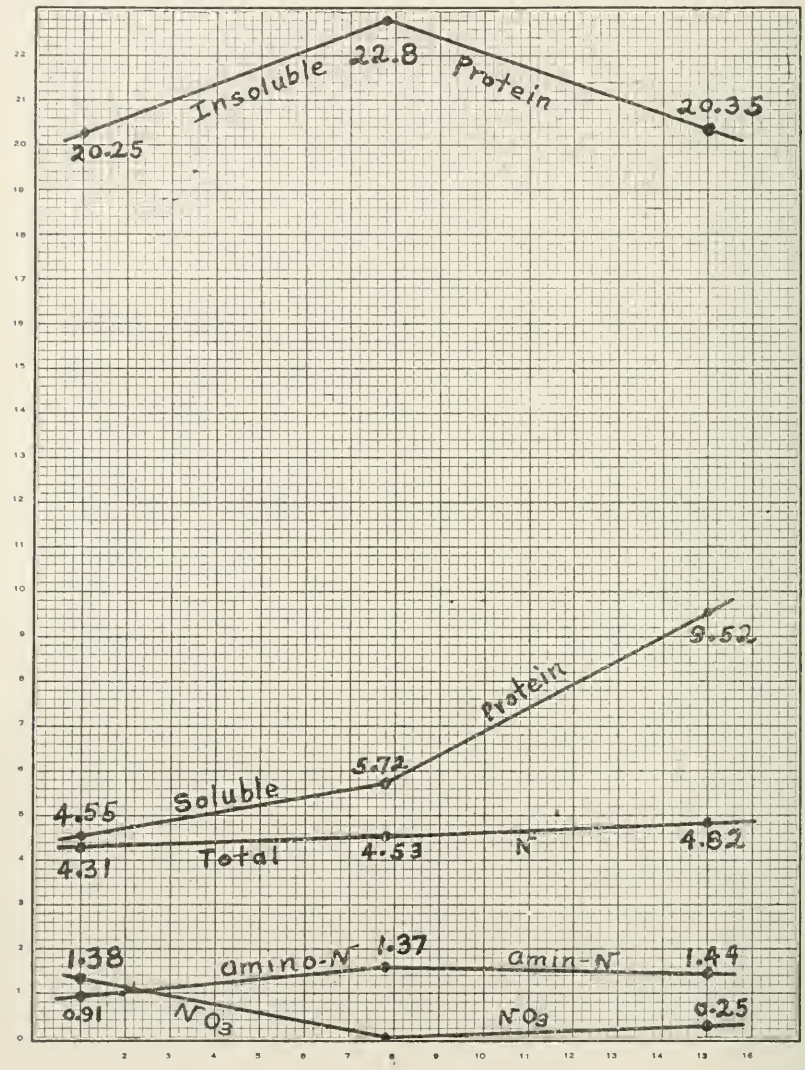

FIG. 5.—Nitrogen compounds in leaves

tion for nitrates. (Previously zinc was used, but this did not reduce any nitrate. Experiment with a known solution 0.5 per cent KNO_3.

per official method by the use of salicylic acid and sodium thiosulphate, reduced only 60 per cent of the nitrate into ammonia.)

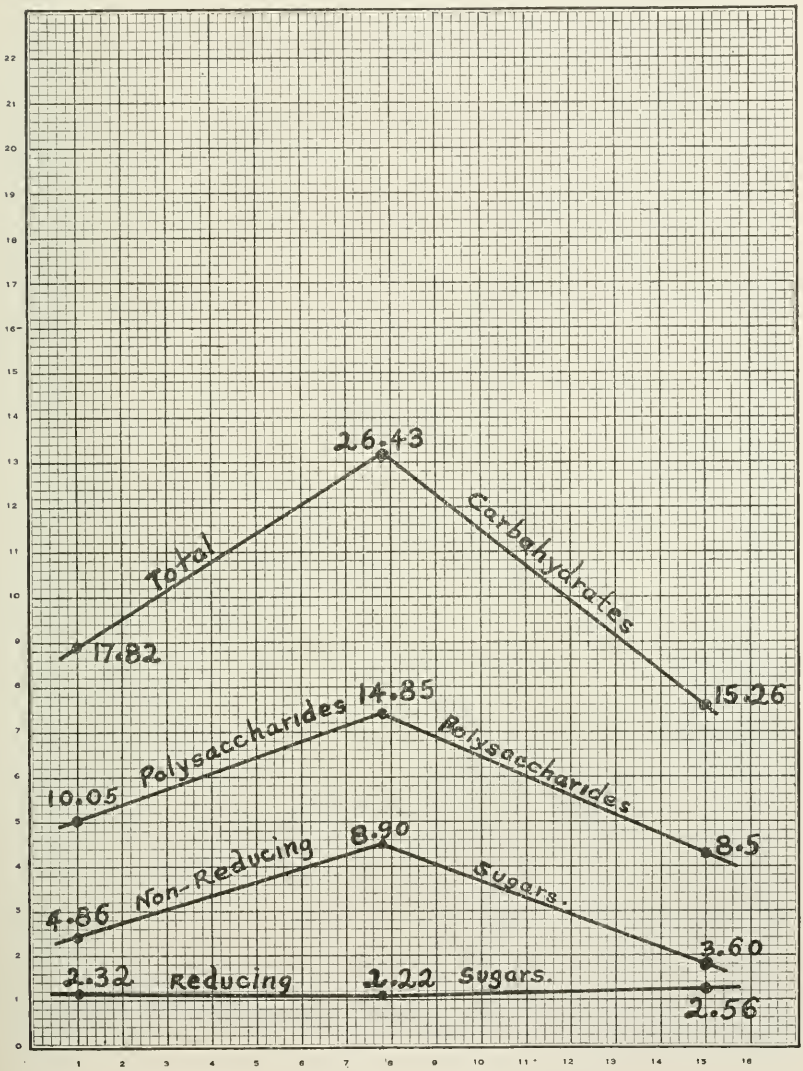

FIG. 6.—Different carbohydrates in roots

It is incorrect to call all this nitrogen as derived from protein, because part of the soluble nitrogen was from the breaking down

of the chlorophyll; but for comparison it is not out of place to calculate the N by the factor 6.29 to convert it into soluble protein

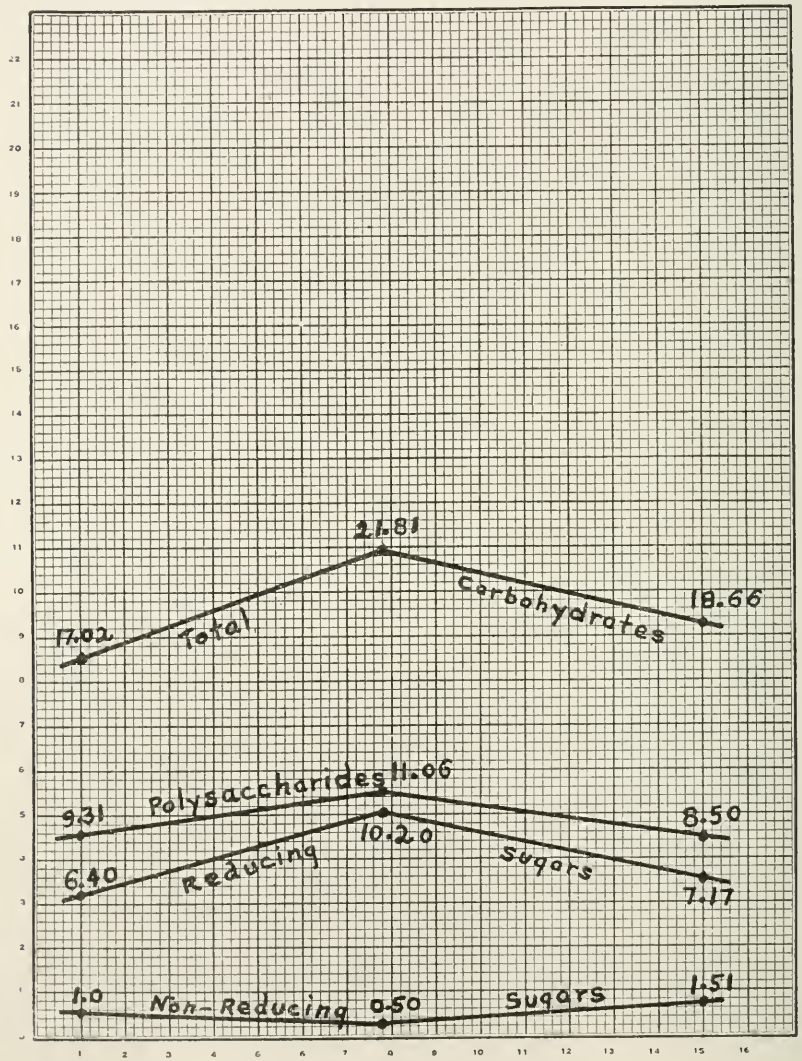

FIG. 7.—Different carbohydrates in stems

for temporary convenience in interpreting the results. The curves of the soluble protein in figs. 3–5 are self-explanatory, showing the variation throughout the season.

CARBOHYDRATES.—The carbohydrates were determined by the reduction method with Fehling solutions. The cuprous oxide

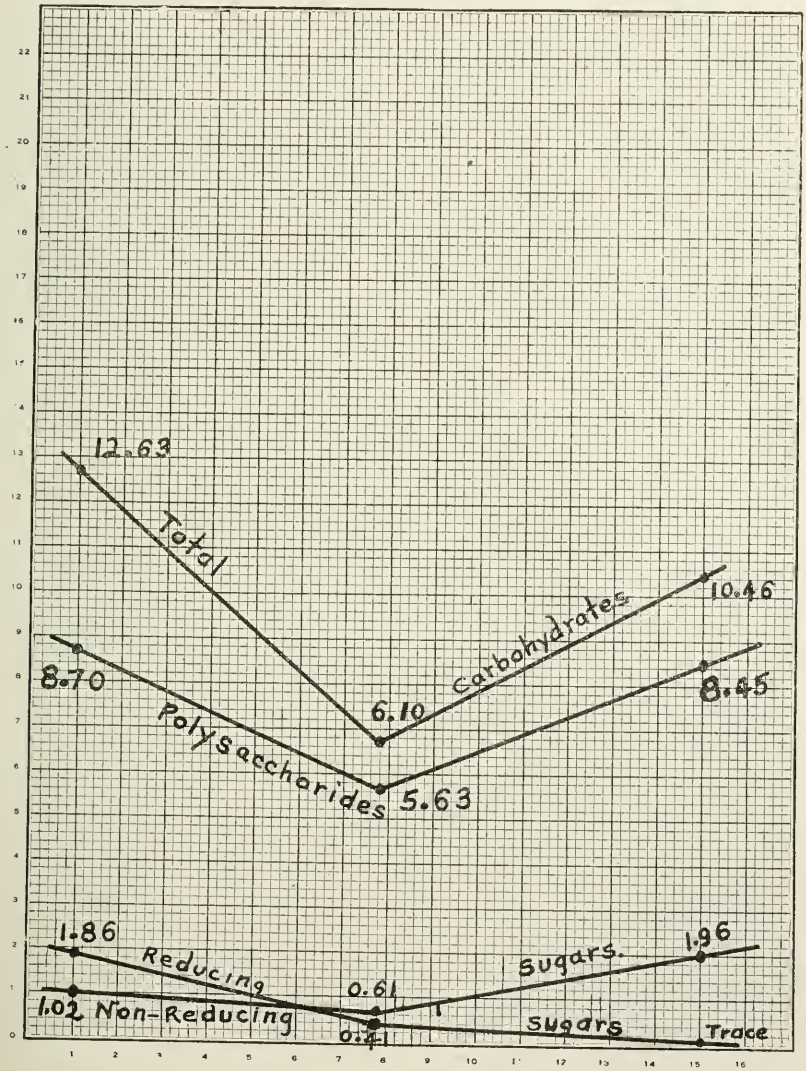

FIG. 8.—Different carbohydrates in leaves

obtained was dissolved in excess of ferric ammonium sulphate with H_2SO_4 previously added. The ferrous ions produced by the oxidation of cuprous oxide were titrated against a N/20 $KMnO_4$

solution, in which 1 cc. represents 3.1 mg. of copper. The corresponding equivalents of the different sugars expressed in milligrams were found in the Munson-Walker table. The weight of sugar found divided by the material used gives the amount of sugar contained in 1 gm. of material.

The soluble carbohydrates are in F_1 and F_2. The reducing sugar was first determined. The non-reducing sugar was obtained by subtracting the reducing sugar from the total sugar by hydrochloric acid hydrolysis at 67–69° C. for 10 minutes.

The insoluble carbohydrates are in F_3. They consist essentially of colloidal polysaccharides, the greater part of which was starch. The polysaccharides were determined by the Fehling solution after acid hydrolysis for 2.5 hours with a reflex condenser.

TABLE III

THE CARBOHYDRATES IN THE GREEN PLANT

Material	Roots		Stems		Branches		Leaves	
	June 3 collection (1–4 inches)							
Total carbohydrates	17.81	17.83	16.95	17.09	....	.....	12.76	12.57
Reducing carbohydrates	2.38	2.27	6.51	6.30	....	.....	1.86	1.87
Non-reducing	5.43	5.46	1.16	1.44	....	.....	2.14	2.05
Polysaccharides	10.00	10.10	9.28	9.35	....	.....	8.76	8.65
	June 20 collection (6–8 inches)							
Total carbohydrates	26.29	26.57	21.78	21.84	....	.....	6.78	6.61
Reducing carbohydrates	2.24	2.19	10.25	10.15	....	.....	0.44	0.38
Non-reducing	9.30	9.43	0.53	0.57	....	.....	0.63	0.67
Polysaccharides	14.75	14.95	11.00	11.12	....	.....	5.71	5.56
	July 8 collection (20 inches)							
Total carbohydrates	15.32	15.21	18.69	18.63	15.18	15.07	10.51	10.41
Reducing carbohydrates	2.85	2.54	7.15	7.20	5.00	5.10	Trace	Trace
Non-reducing	3.82	3.71	1.67	1.50	1.18	1.02	1.96	2.01
Polysaccharides	8.92	8.96	9.87	9.93	9.00	8.95	8.55	8.41

The percentage of the different carbohydrates of various organs estimated at different times throughout the growth period is tabulated in table III. Curves showing the changes in different sugar content in these organs are given in figs. 6–8. These curves show that in the roots the reducing sugars remain constant, while the

non-reducing sugars fluctuate throughout the season. This is just the reverse of what is found in the stems. In the leaves the

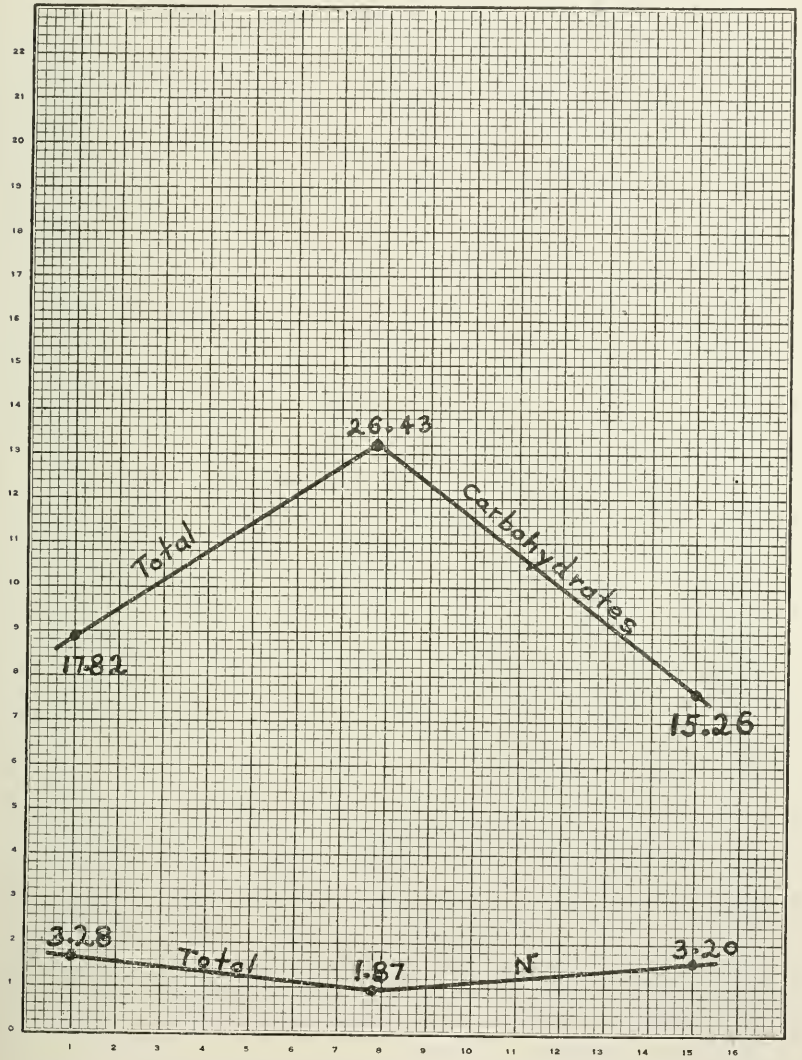

FIG. 9.—Reciprocal fluctuation of carbohydrate and nitrogen in roots (cf. figs. 3 and 6).

fluctuation of the reducing and non-reducing sugars is in the opposite direction; when the reducing sugars are high, the non-reducing

sugars are low, and the reducing type falls to zero at the time of seed formation.

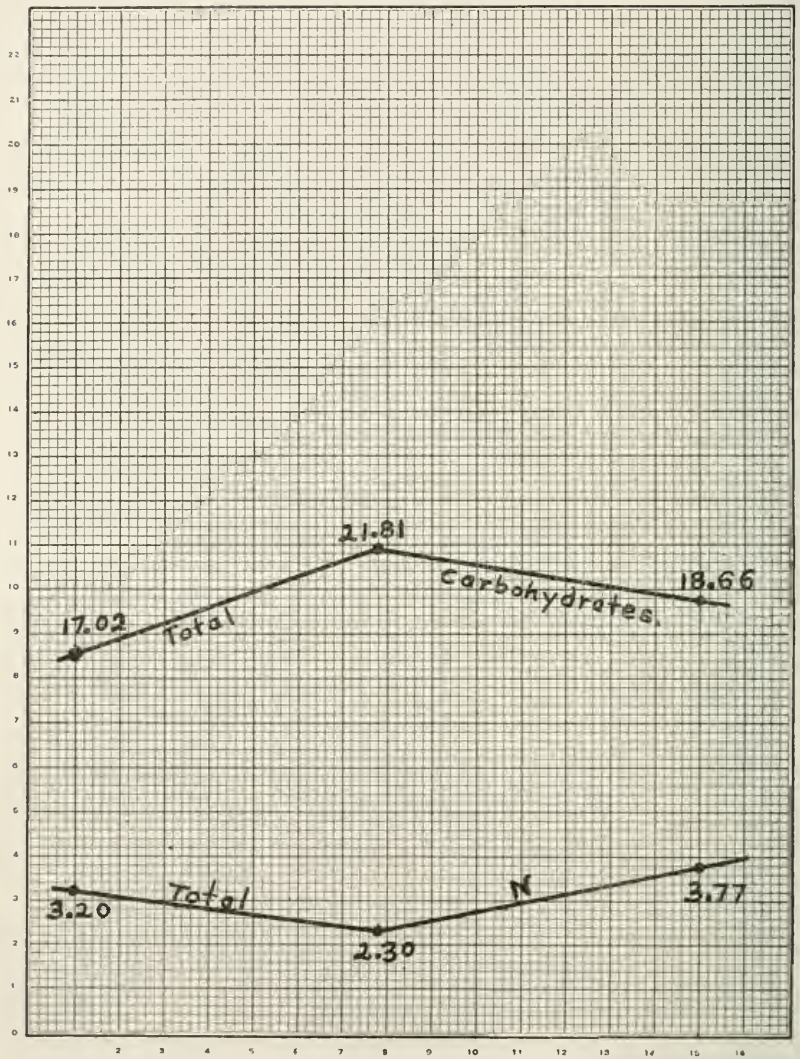

FIG. 10.—Reciprocal fluctuation of carbohydrate and nitrogen in stems (cf. figs. 4 and 7).

CARBOHYDRATE-NITROGEN RATIO.—According to the work of KRAUS and KRAYBILL on the tomato, high nitrogen in a plant is

accompanied by low carbohydrate. "Whatever the conditions under which a plant has been grown, considering the whole plant

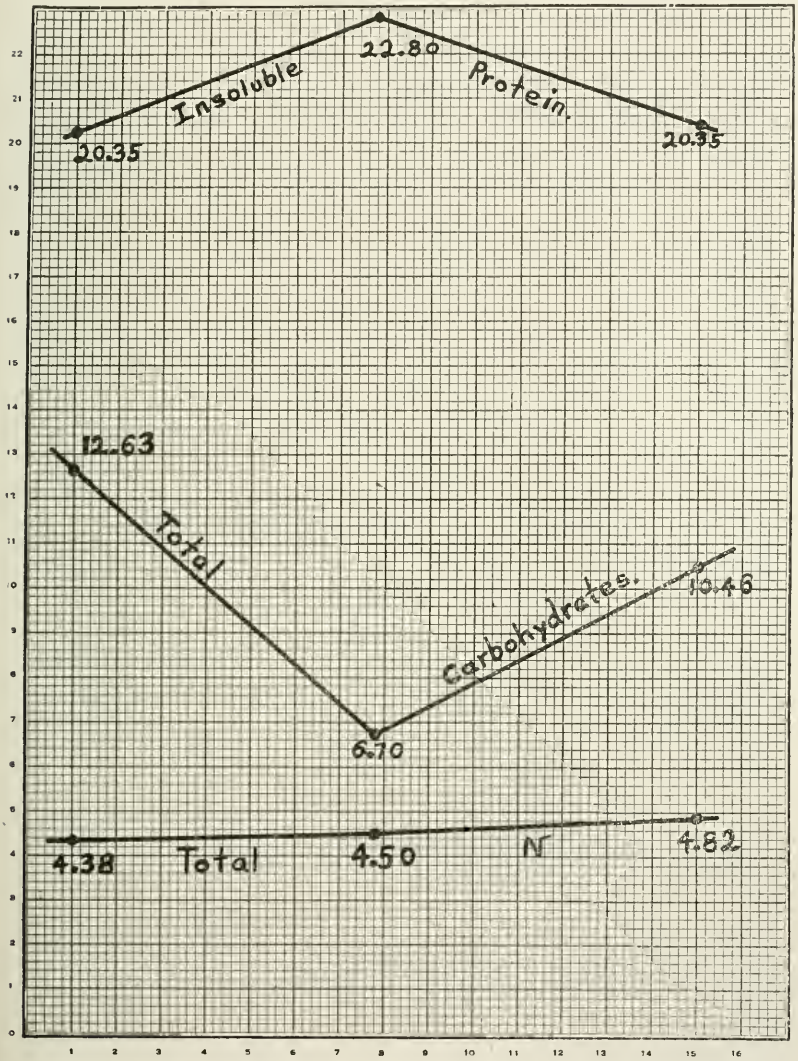

FIG. 11.—Reciprocal fluctuation of carbohydrate and nitrogen in leaves (cf. figs. 5 and 8); notice that carbohydrate reciprocates with insoluble protein and not with total nitrogen.

as a unit, increased total nitrogen and more particularly increased nitrate nitrogen are associated with increased moisture and

decreased free-reducing substances, sucrose, polysaccharides, and total dry matter."

In the work with *Amaranthus* plants I have found a similar situation so far as the relation between nitrogen and carbohydrate is concerned; that is, low nitrogen is accompanied by high carbohydrate and high nitrogen by low carbohydrate. Upon computing the reciprocal condition in the different fractions I find that the product of carbohydrate by nitrogen is not a mathematical constant, but that it varies considerably, sometimes decreasing as the development of the plant progresses. The product varies least in the stem and roots.

Let C_1, C_2, and C_3 be the carbohydrates and N_1, N_2, and N_3 denote the nitrogen, the sub-numbers representing the time of collection. If the carbohydrate and nitrogen hold a reciprocal relation, then $\frac{C_1}{C_2}=\frac{N_2}{N_1}$, $\frac{C_2}{C_3}=\frac{N}{C_2}$, and $\frac{C_3}{C_1}=\frac{N_1}{C_3}$; by clearing the fractions, $C_1 \times N_1 = C_2 \times N_2 = C_3 \times N_3$, etc., or carbohydrate × nitrogen = constant K. Applying this principle, the following constants are obtained.

Insoluble fraction (F_3)

	June 3	June 20	July 8	Av. K.
Roots	15.7	13.06	10.92	13.23
Stems	16.20	10.62	9.20	12.01
Leaves	28.00	20.4	27.6	25.30

Soluble fractions (F_1+F_2)

Roots	10.10	11.00	6.27	9.12
Stems	9.45	8.97	7.88	8.43
Leaves	2.39	2.97	3.15	2.84

These data show that the carbohydrate-nitrogen ratio is not a constant as we think of a constant in mathematics or physics. In plants where great fluctuation occurs in their substratum throughout different parts of the day and different times in the season, this disparity is no positive evidence that such a ratio does not exist. Secondly, regardless of the exactness of the ratio, this much is true, when the carbohydrates are high the nitrogen compounds are relatively low, and vice versa. Figs. 9–11 show this reciprocal

condition in the roots, stems, and leaves in the various stages of development.

SEEDS

A tissue analysis of the seeds was made in order to discover what important compounds were present and the distribution of these compounds in the various fractions. This gives one a more comprehensive knowledge of the chemical constitution of the plant.

PREPARATION.—The seeds were freed from chaff and cleaned in a breeze until all red seeds were removed and only plump black ones remained. These uniform seeds were then ground in a mortar with the pestle by taking a few at a time. A known quantity (25 gm.) was weighed out in triplicate for alcoholic digestion, and the tissues were fractionated and the carbohydrates, the nitrogen compounds, and the phosphorus determined.

TABLE IV

SEEDS; SUMMARY OF TOTAL CONSTITUENTS

Material	A. blitoides		A. retroflexus		
H_2O Av. 3	.....	9.45	.....	8.61	.....
Total N	2.55	2.42	2.54	2.47	2.37
Total P	4.01	3.93	4.63	4.60	4.65
Total carbohydrates	48.27	48.43	47.22	47.03	47.37
Lipins	4.58	4.44	7.67	7.86	7.78
Ash (ignition)	3.50	3.68	4.27	4.19	4.14

DIFFERENT FORMS OF PHOSPHORUS.—After separating the seeds into fractions F_1, F_2, and F_3, the different phosphorus compounds were estimated in the three fractions. In each case the phosphorus was determined by the Pemberton-Kilgore method (10). The analysis of the different forms of phosphorus is given in table V, and the percentage ratio of these different forms to the total phosphorus is given in table VI. The inorganic phosphorus was estimated by the CHAPIN-POWICK method (4). The amounts obtained from fractions two and three (F_2+F_3) were combined and calculated as total inorganic phosphorus. The soluble organic phosphorus was obtained by subtracting the inorganic phosphorus of fraction two only from total phosphorus in the same fraction (F_2). The

phosphoprotein phosphorus was split off from the insoluble fraction (F_3) by digesting a weighed amount (1–3 gm.) with 1 per cent NaOH solution at 37–40° C. for 48 hours in an incubator, after which the solution was neutralized with acetic acid and made up to volume. The insoluble material was separated from the filtrate by the use of the centrifuge. The filtrate obtained was tested for phosphorus with magnesia mixture. The magnesium ammonium phosphate precipitated out by standing overnight was dissolved, and the phosphorus was reprecipitated as ammonium phosphomolybdate and then titrated against a standard alkali (8). The nucleoprotein phosphorus was estimated by the difference between the total phosphorus minus the phosphoprotein and the inorganic phosphorus in the two fractions (F_2+F_3). The lipin phosphorus was determined by taking an aliquot of the ether soluble fraction (F_1). The ether was first driven off on a steam bath before acid digestion and the percentage of this phosphorus was estimated in the same way.

TABLE V

DIFFERENT FORMS OF PHOSPHORUS (PERCENTAGE P) IN SEEDS

Material	A. blitoides		A. retroflexus		
Inorganic P	0.133	0.137	0.131	0.123	0.132
Lipin P	0.014	0.013	0.019	0.020	0.017
Soluble organic P	0.011	0.012	0.023	0.033	0.034
Phosphoprotein P	1.240	1.340	1.840	1.950	1.790
Nucleoprotein P	2.610	2.430	2.620	2.470	2.670
Total P	4.008	3.932	4.633	4.596	4.649

TABLE VI

RATIO OF DIFFERENT P TO TOTAL P (PERCENTAGE P) IN SEEDS

Material	A. blitoides		A. retroflexus		
Inorganic P	3.32	3.49	2.83	2.65	2.84
Lipin P	0.35	0.33	0.41	0.44	0.37
Soluble organic P	0.28	0.31	0.49	0.72	0.73
Phosphoprotein P	31.00	34.10	39.74	42.40	38.50
Nucleoprotein P	65.30	61.80	56.60	53.70	57.50
Total P	100.25	100.00	100.07	99.91	99.94

Tables V and VI show the percentage of the different forms of phosphorus. The data in these tables show that about 96 per cent

of the total phosphorus is in the organic combination in the seed, existing (perhaps) as phosphoprotein and nucleoprotein phosphorus. Both of these forms are insoluble in water, alcohol, ether, and alcohol-water solvents. The inorganic phosphorus is relatively low, and the writer believes that the figures given for inorganic phosphorus in table V are even too high, because the greater part of the inorganic phosphorus was obtained from the insoluble fraction F_3 (4). Moreover, there is no proof that the reagents used did not break down some of the organic phosphorus. The lipin phosphorus is very low, varying from 0.014 per cent in *A. blitoides* to 0.019 in *A. retroflexus*, calculated on dry weight basis. It is interesting to know that in all cases the different forms of phosphorus are relatively higher in *A. retroflexus* than the corresponding forms in *A. blitoides*.

TABLE VII

DIFFERENT NITROGEN COMPOUNDS IN SEEDS (PERCENTAGE DRY WEIGHT)

Material	A. blitoides		A. retroflexus		
Total N	2.550	2.420	2.540	2.470	2.370
Nitrates NO_3	0.200	0.212	0.194	0.193	0.205
Amino N	0.096	0.095	0.089	0.090	0.090
Lipin N	0.027	0.027	0.031	0.032	0.033
Soluble proteins	2.260	2.270	2.660	2.790	2.890
Insoluble proteins	12.640	12.450	12.700	12.820	12.250
Total proteins	14.900	14.720	15.360	15.610	14.140

TABLE VIII

RATIO OF DIFFERENT N TO TOTAL N (PERCENTAGE DRY WEIGHT)

Material	A. blitoides		A. retroflexus		
Total insoluble N	83.20	82.00	80.40	79.00	77.00
Total soluble N	16.97	17.93	19.62	21.01	23.23
Total	100.17	99.93	100.02	100.07	100.23
Nitrate N	1.77	1.88	1.73	1.77	1.97
Lipin N	1.06	1.13	1.24	1.29	1.41
Amino N	4.68	4.65	3.50	3.66	3.84
Other soluble organic N	12.29	13.28	16.12	17.41	19.37

NITROGEN COMPOUNDS.—The distribution of nitrogen in the different fractions of the seeds is about the same as that of phosphorus. The insoluble nitrogen comprised 80–83 per cent of the total nitrogen (tables VII and VIII). The soluble fractions

contain only 17–20 per cent of the total, and most of it exists as organic nitrogen. The portion representing inorganic nitrogen is the nitrate nitrogen, which is relatively small. Calculated as nitrates (NO_3), the seeds contain 0.20 per cent, and this is equivalent to 1.80 per cent of the total nitrogen (tables VII and VIII). The lipin nitrogen is very small, only 0.027 per cent in *A. blitoides* and 0.032 per cent in *A. retroflexus*. These represent 1.10 and 1.31 per cent respectively of the total nitrogen content in these two seeds. In general a high percentage of insoluble phosphorus is accompanied by a high percentage of insoluble nitrogen, and a low percentage of soluble phosphorus by a low percentage of soluble nitrogen.

CARBOHYDRATES.—The polysaccharides are the predominating sugars in these seeds. *A. retroflexus* seeds contain 46 per cent and *A. blitoides* 47.75 per cent polysaccharides (on dry weight

TABLE IX

CARBOHYDRATES IN SEEDS (PERCENTAGE DRY WEIGHT)

Material	A. blitoides		A. retroflexus		
Lipin sugars	None	None	None	None	None
Reducing sugars	None	None	None	None	None
Non-reducing sugars	0.67	0.68	1.12	1.13	1.17
Polysaccharides	47.00	47.75	46.10	45.90	46.20
Total	48.27	48.43	47.22	47.03	47.37
	Ratio of different sugars to the total carbohydrates				
Non-reducing	1.38	1.40	2.37	2.40	2.47
Polysaccharides	98.65	98.60	97.60	97.60	97.60
Total	100.03	100.00	99.97	100.00	100.07

basis). If these sugars are calculated on the dry basis of the total sugars, the polysaccharides represent 97.60 and 98.60 per cent respectively in these two species. A striking contrast is seen on comparing the amount of polysaccharides in the green plant organs (figs. 6–8), which vary only slightly throughout the growing period, with that found in the seeds. In the growing period the highest percentage of polysaccharides was only 14.85, while that of the seeds was 47. In addition to this noticeable contrast, the soluble

sugars, both reducing and non-reducing, were comparatively high in the green plant organs (6–8 per cent), while those of the seeds are low (0.67–1.14 per cent).

LIPIN FRACTION.—The percentage of this fraction is 4.5 for *A. blitoides* and 7.78 for *A. retroflexus*. A closer examination of this fatlike substance shows that it contains phosphorus and nitrogen. The percentage of this nitrogen and phosphorus is very low (calculated on dry weight basis of whole sample). The presence of nitrogen and phosphorus indicates that the seeds contain phosphotides. The atomic ratio of nitrogen to phosphorus was determined by dividing the percentage by their respective atomic weights. The atomic ratio of N:P for *A. blitoides* is 1:2.3, and that for *A. retroflexus* is 1:2.6. This shows that other forms of phosphorus must be present than that existing in "ideal lecithin," which implies that the atomic ratio of N:P=1:1 (5, 13).

In addition to lecithin, a phytosterol was present in the seeds. This could not be quantitatively determined by using animal cholesterol as a standard, because animal cholesterol (17) has a different tint from that of the plant. BUCHARD'S color reaction gives a deep blue color for animal cholesterol, while for the seeds it gives a yellowish green. The amount of phytosterol in these two species of seeds was compared. Assuming that the phytosterol in *A. blitoides* is unity, that in *A. retroflexus* is 2.8.

TABLE X

CONSTITUENTS OF LIPIN FRACTION (F_1) OF SEEDS (PERCENTAGE DRY WEIGHT)

Material	A. blitoides			A. retroflexus		
Lipin, fats, etc. (wet basis)	4.15	4.07	4.23	7.02	7.18	7.09
Lipin (dry basis)	4.58	4.44	4.66	7.67	7.86	7.78
P in F_1	0.300	0.300	.....	0.240	0.260	0.220
P calculated in whole sample	0.014	0.014	.....	0.019	0.020	0.017
N in F_1	0.600	0.640	.....	0.402	0.400	0.420
N calculated in whole sample	0.027	0.027	.....	0.031	0.031	0.033

INORGANIC ELEMENTS.—The 1917 seeds of *Amaranthus retroflexus* were used for the estimation of the inorganic elements. The percentage of total ash given in table IV is 3.59 per cent for *A. blitoides* and 4.20 for *A. retroflexus*, but this is actually too low

for the inorganic elements present in the seeds, because the total phosphorus alone is 4.0 and 4.60 per cent respectively for the two species. This discrepancy is due perhaps to the loss of nitrates and part of the sodium, potassium, etc., in burning in the electric muffle. In the following analysis of the inorganic elements acid digestion (concentrated H_2SO_4+concentrated HNO_3) was used for the kations and alkaline fusion for the anions. The potassium was determined directly in the presence of all other elements except ammonia (NH_4−ion) and strong acid, as $K_2NaCO(NO_2)_6-\frac{1}{2}H_2O$ (11). Magnesium was estimated by the volumetric method as NH_4MgAsO_4 (7). The percentage of inorganic elements is as follows:

INORGANIC SALTS (*A retroflexus*); 1917 SEEDS

	Percentage	Percentage
Silica	0.42	0.40
$Al_2O_3+F_2O_3$	0.53	0.56
CaO	0.54	0.53
MgO	0.82	0.84
K_2O	0.38	0.35
Na_2O		no determination
Cl	Trace	Trace
SO_3	0.34	0.33
P_2O_5	8.90	8.85
N_2O_3 (nitrates)	0.12	0.123
Total (not including Na_2O)	12.05	11.98

Discussion

In spite of the inaccuracy of BOUTIN'S method for the determination of the nitrates, his results are probably not far from correct. He stated his results in percentage of KNO_3 as shown in the second column of table XI. I have calculated these as percentage of NO_3, as shown in the third column. His percentages of nitrates are not far from those found by me for the stems (8.57 per cent) and branches (12.50 per cent) of *A. retroflexus*, July 8 collection, as shown in table II.

	Percentage KNO	Percentage equivalent in NO
A. retroflexus	22.77	13.92
A. blitum	11.68	7.17
A. ruber	16.0	9.82

Table XI shows the results of the analyses of the seeds by various authors. In the main there is fairly close agreement, but in some cases there are considerable discrepancies. The discrepancies can probably be explained by the different chemical methods used by the various authors and by the lack of uniformity in the different crops analyzed.

TABLE XI

COMPARISON OF SOME OF THE ANALYSES ON *Amaranthus* SEEDS

MATERIAL	PAMMEL AND DOX		HARDING AND EGGE			WOO	
			A. retroflexus				
	A. blitoides	A. retroflexus	20 mesh	72 mesh	Oven dry	A. blitoides	A. retroflexus
Condition of seeds			Non-	uniform		Matured uniform	Matured uniform
						Av. of 3	Av. of 3
H_2O				11.28	8.60	9.45	8.61
Lipins (fats)	Little*	Little*		7.92	8.46	4.56	7.77
Polysaccharides	Abundant	Abundant	39.77	40.98	44.83	47.68	47.21
Reducing sugars			Trace	Trace	Trace	None	None
Non-reducing sugars (sugar after inversion)			2.08	2.15	2.35	0.67	1.14
Nitrogen	1.88	2.49				2.48	2.46
Protein	11.75	15.59	18.57	19.13	20.93	14.81	15.03
Ash			4.33	4.46	4.88	3.59	4.20

* Microchemical test.

From the results of this study it would seem that *Amaranthus retroflexus*, and probably other species of the same genus, can bear, as they ordinarily do bear, large amounts of free nitrates without being forced out of reproduction into extreme vegetation. This genus apparently is endowed with a very high capacity for nitrate absorption, as well as for maintaining its full seed production power in the face of a great excess of free nitrates. In this respect it seems to differ from the tomato studied by KRAUS and KRAYBILL, and probably from many other plants. Considering all angiosperms, it is likely that, due to hereditary characters, there is a great range of ease with which plants can be forced to excessive vegetation by extreme nitrate supply within the plant. It is well known that a given level of fertility that will throw small grains into extreme straw production with deficiency of grain will give excellent grain production in corn. This may be due to the lower nitrate absorbing power of the corn, to its greater

photosynthetic activity to balance the nitrates absorbed, or to the higher carbohydrate-nitrogen ratio accompanying best grain production. Which of these three possibilities really determines the situation can only be answered by such studies as those made or suggested on the tomato by KRAUS and KRAYBILL, or studies of the type made in this paper. It is evident, however, that there is need of numerous studies of the carbohydrate-nitrogen ratio in plants, both in regard to the factors affecting this ratio and the effect of the ratio on plant characters. As was suggested in the review of the literature at the beginning of this article, such studies are likely to throw much light on other physiological features than vegetation and reproduction.

Summary

1. There is a large amount of nitrate in the organs of *A. retroflexus*. The stem and branches are the primary nitrate storage organs. The rate of nitrate absorption increases with the aging of the plant, perhaps partly being due to the development of the root system with numerous branching rootlets, increasing the radius of the feeding area from a few inches to 2 ft. or more.

2. This high capacity for nitrate absorption and storage must be an important factor in making *Amaranthus* a very successful competitor against cultivated plants, so effectively withdrawing as it does the nutrient element most commonly limiting plant production. It would be interesting to know how generally and to what degree weeds possess this power.

3. The carbohydrates and nitrogen compounds fluctuate throughout the growing period. The fluctuation of the carbohydrates is in the reverse order of the nitrogen compounds. This inverse ratio is not a truly mathematical constant, but in general when the carbohydrates are high the nitrogen compounds are low, and vice versa. As the nitrate nitrogen composes more than 50 per cent in the stems and branches, there is a possibility that nitrates have some modifying effects on this reciprocal relationship. This inverse ratio is due partly to the synthesis of protein, chlorophyll, phospholipin, and other organic nitrogen compounds at the expense of the soluble carbohydrates.

4. Tissue analysis of the seeds shows the distribution of different forms of phosphorus in the various fractions. The organic phosphorus, which consists chiefly of phosphoprotein and nucleoprotein phosphorus, is high, and that of the inorganic form is low.

5. The distribution of nitrogen in seeds is in the same order as that of the phosphorus. The insoluble portion contains 80–83 per cent of the total. The soluble part varies from 17 to 20 per cent, most of which is in the organic form. The inorganic form is represented by the nitrate nitrogen.

6. The predominating sugars in the seeds are the polysaccharides. These compose nearly one-half of the total dry weight of the seeds. In both *A. retroflexus* and *A. blitoides* there is absence of lipin sugars in F_1 and reducing sugars in F_2. Only a small amount of non-reducing sugars was present in the two varieties.

7. The presence of nitrogen and phosphorus in the lipin fraction indicates that the seeds contain phosphatides. Phytosterol was also present. By comparison, *A. retroflexus* has 2.8 times as much as *A. blitoides*.

I am indebted to Drs. Wm. Crocker, S. H. Eckerson, and F. C. Koch for their kind advice and aid during the progress of the work.

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STAMINATE STROBILUS OF TAXUS CANADENSIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 255

(WITH PLATES XXIV-XXVI AND TWENTY-TWO FIGURES)

A. W. DUPLER

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Introduction

In a previous paper (8) the writer described the gametophytes of *Taxus canadensis* Marsh., with the statement that other phases of the morphology would be treated in later papers. In this paper the staminate structures with respect to development and vascular anatomy are described. The lack of detailed information concerning these structures has seemed to the writer sufficient justification for the investigation here reported. In view of the generally recognized conservative character of the staminate structures in conifers, it seems that a more extended investigation of them, in the group as a whole, would be worth while. The description of the ovulate structures will be given in another paper.

The general statement in the previous paper as to material and methods will also apply here. The writer is under obligations to Professor W. L. EIKENBERRY, of the University of Kansas, for some material collected in northern Illinois a number of years ago. Acknowledgments are also due Professors JOHN M. COULTER and C. J. CHAMBERLAIN, under whose direction the study of *Taxus canadensis* was begun.

Historical

While the male gametophyte and its attendant features have received considerable attention, apart from the general more obvious features very little is found in the literature dealing with *Taxus* as to the morphology of the staminate strobilus itself. The earlier workers who studied the staminate structures of conifers were concerned largely in attempts to interpret them in terms of the angiosperm flower, naturally leading to confusion as to the true nature of the structures. These earlier views have been summarized by VON MOHL (26) in perhaps one of the most important

of the early papers dealing with the "male flowers" of conifers, and to which we are indebted for part of the following statement regarding this early interpretation of the staminate strobilus of *Taxus*.

LINNAEUS (16) regarded the entire strobilus as a single flower, with the stamens in a cylinder, the perianth lacking and replaced by bud scales. JUSSIEU (13) held that the strobilus was a monadelphous flower; while LINDLEY (15) considered the strobilus as a true cone with naked monadelphous flowers, each sporophyll representing a flower. RICHARD (19) went still farther, with the rather unique view that there were 5–8 flowers under each scale to which the stalk of the flower is attached on the underside. According to this view the pollen sac represented a "flower," and he had a similar interpretation for the sporophylls of *Thuja* and *Juniperus*. ZUCCARINI (28), regarding the reproductive structures as modified portions of the stem, comparable with the phylloclads of *Phyllocladus*, described the anther of *Taxus* as 7–8-lobed around the tip of a central column. He considered that *Taxus* has the most complete male flower in conifers, in other forms the anther folds growing on only one side of the central column, the other side growing out into a scale. VON MOHL opposes the idea of the stem character of the "flower" of conifers, and objects to the view that the "anthers" of other conifers have been derived from such a structure as that of *Taxus*, because "we have yet no certain data with which we can determine with certainty whether the anther of *Taxus* arises from one leaf or from a whorl of leaves."

As compared with our present ideas these early views are rather strange, having largely only a historical interest, with very little bearing on the real morphology of the structures concerned. Considerably later STRASBURGER (22) made some observations on *T. baccata*, describing the spiral arrangement of the scales of the strobilus and the grosser features of the development of the sporophylls. He held that the peltate stamen of *Taxus* represents the "extreme form of stamen," and found that it begins as a rounded knob about the first of August, becomes lobed by lateral swellings due to internal growth, and that pollen mother cells form in these lateral swellings and produce pollen by tetrad divisions. He also describes the pollen region as separated from the epidermis by two

layers of irregular cells, and states that dehiscence is accomplished by the rupture of cells at the base and sides of the pollen sac. CHAMBERLAIN (3) describes the microsporangium of *T. canadensis* at the mother cell stage (October 1, 1897), at which time the nuclei are still rather small in comparison with the size of the cell, the tapetum being sharply differentiated, and its cells showing no tendency to plasmolyze like the cells of the sporangium wall. PILGER (18) describes the general external features, largely from the taxonomic viewpoint, speaking of the "flower" as consisting only of sporophylls surrounded at the base by a scale envelope which completely incloses the flower in the bud state. He regards the "leafy structure" of the anther, which is yet to be recognized in *Torreya* and *Cephalotaxus*, as being "entirely lost" in *Taxus*.

In the related forms the staminate structures of *Torreya* have been described in a general way by PILGER, based on *T. nucifera;* in more detail by Miss ROBERTSON (20) for *T. californica; and* by COULTER and LAND (5) for *T. taxifolia.* In *Cephalotaxus* some of the features of the spermatogenesis have been described by STRASBURGER (23), and by ARNOLDI (1). STRASBURGER (24) pointed out that the pollen grain divides in the sporangium before shedding; LAWSON (14) also confirms this in *C. drupacea;* and WORSDELL (27) gives a description of the general features of the "male flower," based on *C. Fortunei*, comparing it with those of other forms (*Phyllocladus* and *Ginkgo*), especially in the sporophyll features.

Strobili buds

In the axils of the leaves of the shoot of a given season 3 types of structures are produced: (1) the vegetative buds from which develop the lateral leafy shoots of the next season; (2) the young staminate structures, maturing the next season; and (3) the ovule-bearing structures, also maturing the next season. During the first season all of these structures are in bud form, the staminate buds during the latter part of the summer and winter being more globular than the other two kinds, which are so nearly alike in external appearance as to make their distinction uncertain except by very careful examination.

The rudiments of these structures begin to develop very soon after the beginning of growth of the terminal bud in the spring, the

FIGS. 1–6.—Longitudinal sections of young buds: fig. 1, young vegetative shoot with bud rudiment in axil of young leaf; fig. 2, vegetative bud with conical apex; fig. 3, staminate bud with broadened apex; fig. 4, ovulate bud, showing vegetative tip (to left) and rudiment of ovulate strobilus in axil of scale; fig. 5, young staminate strobilus, showing primordia of stamens and tip of axis; fig. 6, young staminate strobilus with primordia of sporophylls, axis apex not evident; ×36.

rudiments appearing as conical projections in the axils of young leaves (fig. 1). By the middle of June these axillary structures have

attained a length averaging about 1.5 mm., consisting of the main axis surrounded by compactly arranged scales. In this early stage one cannot distinguish these structures from one another, either by external appearance or in section. Early in July, however, one can recognize in median longitudinal sections the beginning of the differentiation which is now taking place, the apex of the vegetative bud remaining conical (fig. 2), as is characteristic of the vegetative stem tip (fig. 1), the apex of the staminate structure becoming broadened (fig. 3), while the ovule-bearing structure is recognizable by the rudiment of the ovulate strobilus appearing in the axil of one of the scales near the tip of the primary shoot (fig. 4). All 3 kinds of buds may occur on the same shoot; in fact, this is the usual occurrence, with the staminate buds generally the more numerous, the vegetative buds nearest the tip, and one to several ovulate buds a short distance below the vegetative ones, the staminate buds occupying the older portion of the shoot.

The buds arise only on the current season's growth, and in case of the staminate structures always mature the next season. No cases were observed in which staminate strobili were produced on older growth, nor were any cases found in which the buds remain dormant for a time and then mature. Miss ROBERTSON, in her study of *Torreya californica* from trees growing in England, found that while the staminate strobili are formed in the axils of the leaves of a current season, they may remain dormant for as long as 3 years. In *Taxus* buds may be found on older growth, but they are either dormant vegetative buds or persisting primary shoots of the ovuliferous structures of a former season, as will be described more fully in the paper dealing with these structures.

Sporophylls

PRIMORDIA

The broadened apex (fig. 3) is the first indication of the true nature of the staminate strobilus bud, and can be recognized first about July 1. STRASBURGER (22) was able to recognize the staminate structure of *T. baccata* about August 1, and in *Torreya taxifolia* COULTER and LAND (5) first observed the staminate buds in July,

but the primordia of the sporophylls did not begin to appear until August. The greater meristematic activity in some regions of this rounded apex than in others marks the position of the primordia of the sporophylls. These soon become rounded lobes above the general surface (figs. 5, 6). The nature of the growth of the primordium would indicate that it arises from a group of meristematic cells rather than from a definite initial; at least no defined sporophyll initial could be recognized.

The sporophylls are probably spirally arranged, although this is somewhat indefinite, and indications were found in a few cases that they may arise in acropetal succession (fig. 5); but if this is the case it is very soon obscured in the uniform development of the primordia as the sporophylls develop, no trace of the axis apex being recognizable after the very early beginnings of the sporophylls. The early development of the primordium is uniform in all directions from its central axis, at least until the differentiation of the archesporial initials takes place. The strobilus in this stage shows a series of rounded sporophyll primordia (figs. 5, 6, 23). The later development of the sporophyll is so intimately bound up with the development of the sporangia as to best be described in connection with them. In fact, the development of the sporangia determines the shape and character of the sporophyll, as aside from the sporangia the sporophyll consists of practically nothing excepting the short central axis and the epidermis.

MICROSPORANGIUM

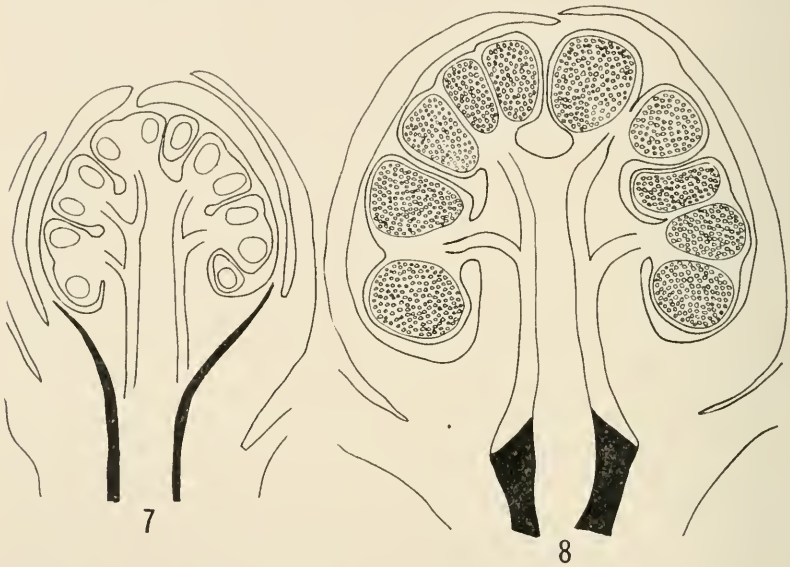
Archesporial initials.—Hofmeister (11) seems to have been the first to publish with reference to the microsporangium of conifers, reporting the spore mother cell stage as being reached in *Pinus maritima* in November. Goebel (9) traced the archesporium of *Pinus* to a single hypodermal cell, and claimed a similar origin for the archesporium of *Thuja*. His most important observation on this point was that the development of the microsporangium is like that of the eusporangiate ferns. Coker (4) in *Taxodium distichum*, and Nichols (17) in *Juniperus communis* var. *depressa*, also found a hypodermal origin of the archesporium, in the latter case consisting of "a plate of radially elongated cells, 4–6 in

number, when viewed in longitudinal section." COULTER and LAND, interpreting the structures in abortive sporangia, conclude that in *Torreya taxifolia* there is "a single hypodermal archesporial cell." Miss ROBERTSON did not get the origin of the sporogenous tissue in *T. californica*.

In *Taxus canadensis* the development follows the usual eusporangiate method, the 4–8 (usually 5–7) archesporial initials arising from the hypodermal layer of the sporophyll primordium while this structure is yet quite small (fig. 23), being uniformly distributed along its margin, and, dividing by periclinal walls, form the primary wall cell and the primary sporogenous cell (figs. 27–29), as in *Torreya taxifolia* (5) and most other forms. These initials are first to be recognized by the size of the cells and of their nuclei (figs. 23–26). One initial cell seems to be the rule, although cases were found in which the archesporium consists of 2 cells (fig. 26).

SPOROGENOUS TISSUE.—The primary sporogenous cell or cells soon divide periclinally (fig. 30) or anticlinally before or after the division of the primary wall cell, and by successive divisions the mass of the sporogenous cells is increased (figs. 31–35), the formation and growth of which result in the lobed peltate structure of the sporophyll, the sporangia being uniformly distributed around the central axis which continues the very short stalk of the sporophyll. As the tissue increases there is a corresponding growth of the epidermis and the sporangium wall (to be described later), the completion of which results in the separation of the sporogenous tissue from the other portion of the sporophyll (fig. 34). The tapetum is differentiated from the peripheral layer, and the remaining sporogenous mass increases in amount until the mother cell stage is reached early in October, as described by CHAMBERLAIN (3) and the writer (8). This has been given (6), and even quite recently (7), as the winter condition of the microsporangium, and has frequently been quoted by writers. As the author has already pointed out (8), microspore formation takes place during the early part of October, collections covering a number of years and from several localities in the northern United States bearing out the statement that the microspore is the winter condition of *Taxus canadensis*. STRASBURGER (25) found microspore formation in *T. baccata* taking place

in February in 1904, during unusually warm weather, indicating that in this form the sporogenous tissue remains in the mother cell stage until spring. It would be of interest to know the behavior in the extreme northern part of the range of *T. canadensis*, as it is possible that in regions farther to the north the microspore stage might not be reached before winter. The microspore mother cell stage is the winter condition of *Torreya californica* (20) in England



FIGS. 7, 8.—Median longitudinal sections of older strobili: fig. 7, at time of completion of sporangium wall, showing oval areas of young sporogenous tissue; vascular tissue of axis and upper scales, shown in black, embryonic vascular tissue of upper portion in outline; fig. 8, winter condition of strobilus, showing globular character of bud and microspores; vascular tissue as in preceding figure; ×36.

and of *T. taxifolia* (5) in Florida. During this development the strobilus has grown considerably in size (cf. figs. 7 and 8), becoming more pronouncedly globular, and it remains in this condition until the renewed growth of spring takes place.

No cases of abortive sporangia were found, and it seems a safe assumption that a sporangium develops from each initial or initial group. The adult sporangia show some variation in size, but not enough to indicate any tendency to abortion of any of them. This

is in marked contrast with the behavior in the related *Torreya*, in which a resin cavity results from the abortion of some of the potentially sporogenous tissue of the sporophyll, the abortion beginning at the primary sporogenous cell stage, as pointed out by COULTER and LAND for *T. taxifolia*. This results in the sporangia occurring on only one side of the otherwise peltate sporophyll. Miss ROBERTSON also finds that normally there are 4 sporangia on the side of the sporophyll of *T. californica* and a resin cavity on the other side, but that the strobilus axis sometimes terminates in a radially symmetrical sporophyll, like that of *Taxus*, with 6 or 7 mature sporangia. Whether a resin cavity is present in such a sporophyll is not stated, the inference being that most or all of the sporangium initials reached maturity. A similar abortion of sporangia, in the formation of mucilage cavities, is indicated by Miss STARR'S (21) work on *Ginkgo biloba*. COULTER and LAND find in *Pinus Laricio* resin cavities related to sporangia, exactly as are the lateral sporangia to the two middle ones in *Torreya*, and say "there is evident a tendency to reduce the number of sporangia by abortion, a reduction that has proceeded farther in *Pinus* than in *Torreya*, and in the latter farther than in *Taxus*." It seems that when resin or mucilage cavities are present in the sporophyll the sporangium initials are involved, and when absent these initials may all function normally, as in *Taxus*. Whether this can be made as a general statement for all forms with resin cavities in the sporophyll must wait for more extensive work on other forms. *Cephalotaxus* has a sporophyll similar in general appearance to that of *Torreya*, but it is not known whether any abortion takes place.

SPORANGIUM WALL.—The initial development of the wall is from the primary wall cell, which by a periclinal division forms a tier of 2 cells. As the sporogenous tissue develops, these wall cells divide anticlinally (fig. 31), increasing the extent of the wall layers. Only a portion of the wall is derived from the primary wall cell, however, as other cells abutting the young sporogenous tissue divide periclinally and add to the wall, first on the outer side (figs. 31–33) and then on the inner side as well (fig. 34), thus completely enveloping the young sporogenous tissue. The wall usually consists of 2 layers of cells, although 3 or even more layers may be present,

especially at the angles formed by the mutual pressure of the sporangial lobes.

As the sporogenous tissue increases in size there is pressure upon the wall cells, and they become flattened and extended, so that at the time of their maximum development they are broad thin plates. By the time of spore formation they are usually quite flattened (fig. 36), and during the further growth of the spores become more or less disorganized, so that by the time the spores have reached maturity, just before shedding, the wall has become a very thin layer abutting the epidermis, which has now become, in effect, the functional sporangium wall.

Tapetum.—At the time when the sporangium wall is completed the sporogenous tissue inclosed within it is uniform in appearance (fig. 34). Soon, however, the peripheral layer of this tissue becomes differentiated as a tapetum (fig. 35), thus originating from the sporogenous tissue and not from the inner layer of the sporangium wall, as in some forms. The tapetum, however, has its chief significance from a physiological standpoint, being generally regarded as a nutritive layer, its origin seeming to be of little morphological significance. The tapetal cells are usually uninucleate, but not infrequently are binucleate (figs. 35–36). The tapetum is quite distinct during later phases of the development of the sporogenous tissue, is sharply differentiated at the spore mother cell stage, as pointed out by Chamberlain (3), and remains distinct during the early winter (fig. 36). With the growth of the microspores in the spring it becomes less and less prominent, until near pollination it consists of only a very thin layer of disorganized material surrounding the spore mass.

EPIDERMIS

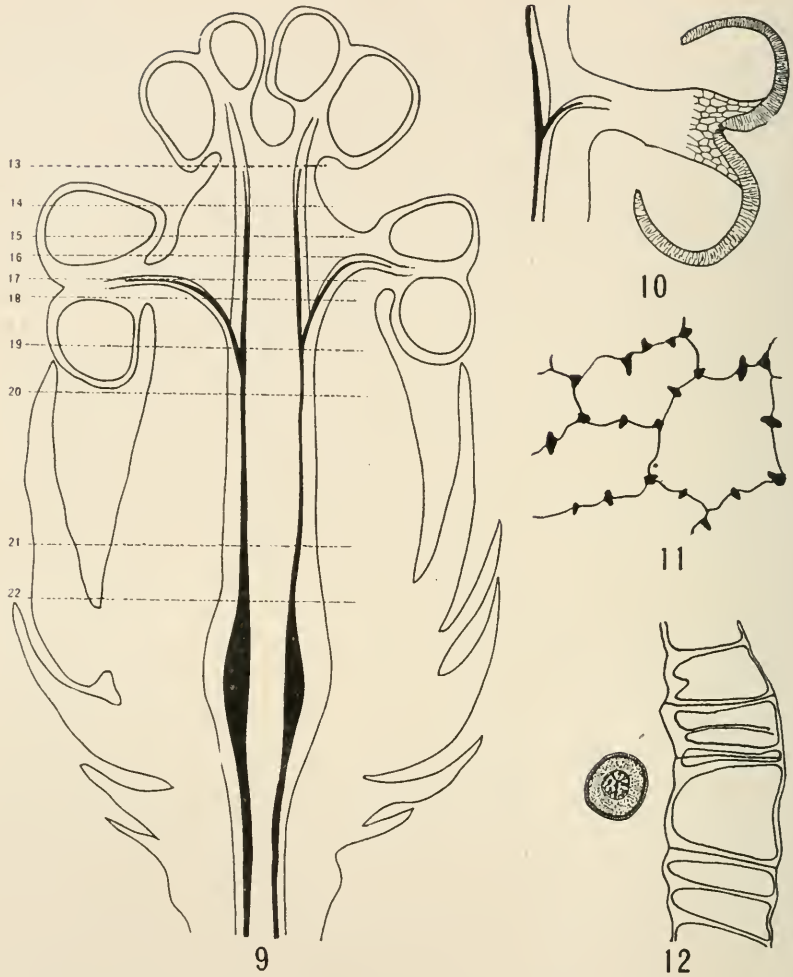
From the beginning of the primordium to the mature sporophyll the epidermis remains as a distinct layer, the sporangium developing from hypodermal tissues, as already stated. During the early growth of the sporophyll the epidermis is meristematic throughout, dividing anticlinally (fig. 24), its surface area thus keeping pace with the increase in the mass of the sporogenous tissue. An occasional periclinal division results in the epidermis becoming 2 cells thick at

some points. The meristematic ability, however, soon becomes limited to the base of the sporangium, the epidermal cells of the remainder of the sporophyll becoming larger and with less dense contents, the cells at the base remaining isodiametric and rich in cytoplasm (figs. 32–34). As the sporangium increases in size, causing a more pronounced lobing of the sporophyll, the necessary increase in epidermal surface is effected by the enlargement of the non-meristematic cells and the addition to them of cells from the basal meristematic region. The enlarged cells become filled with an amorphous substance and the walls become thicker.

By the time the sporangia are mature the epidermis has become the functional wall of the sporangium, owing to the practical disintegration of the true sporangium wall. At maturity the epidermal cells are devoid of contents and have the markings characteristic of the walls of many sporangia (figs. 11–12), these thickenings exercising a hygroscopic effect, useful in the liberation of the spores. Jeffrey (12) regards this thickening of the epidermal cells of the sporophyll, in a mechanical dehiscing device, as the result of the invasion of the epidermis by mechanical tissues of fibrovascular origin. There are no indications in *Taxus* of mechanical elements elsewhere in the sporophyll. Coulter and Land found numerous stomata in the epidermis of *Torreya*. In *Taxus canadensis* there is a single stoma on a sporophyll, at the center of the peltate disk, occupying the bottom of the depression caused by the enlarged sporangia (fig. 10). Goebel (10) shows a similar situation in *T. baccata*.

Mature strobilus

The scales at the base of the strobilus are small and decussate, increasing in size and becoming spiral in arrangement above, the uppermost ones being considerably larger than the lower ones, and function as bud scales in the immature condition of the strobilus. The scales are brownish in color, with heavily cutinized outer epidermal walls, especially on the abaxial surface, the stomata occurring only on the inner surface (fig. 37), reversing the condition on the vegetative leaves of the plant, where the stomata occur only on the lower (abaxial) surface. The midrib is marked by the



FIGS. 9–12.—Fig. 9, median longitudinal section of mature strobilus just before pollen shedding, showing "elongating region" of axis and 4 sporophylls; xylem of bundle black; note that xylem becomes centrally placed in upper portions and does not extend as far into stalks as phloem; numbers at left (13–22) indicate approximately levels of cross-sections of strobilus shown in figs. 13–22; fig. 10, median section of open sporophyll, showing elongated stalk, open sporangia, and solitary stoma in center of depression of disk; fig. 11, tangential section of mature epidermis, showing mechanical thickenings on walls; fig. 12, cross-section of mature epidermis, with microspore, at time of shedding; figs. 9, 10, ×36; figs. 11, 12, ×475.

vascular bundle, when present, and occasionally by sclerenchyma-like cells along the outer margin of the midrib. In the young strobilus the mesophyll of the scale is compact, but as the strobilus matures large air spaces develop. In addition to the solitary stoma found on the sporophyll, stomata occur on the strobilus axis between the bases of the sporophylls with rather surprising frequency, being found only on this portion of the axis and not on the portion between the upper scales and the lower sporophylls. While the functional character of these stomata might be open to question, owing to their position rather than to their structure, their chief interest probably lies in their morphological significance as hereditary structures from a more highly vegetative ancestral strobilus.

During the autumn, winter, and early spring the strobilus has the appearance of a globular "bud," the stamens being surrounded by the uppermost scales (fig. 7). The axis between the upper scales and the bases of the lower sporophylls is very short and remains so until a few days before maturity, during the latter part of April in central Pennsylvania, at which time there is a rapid enlargement and elongation of this portion of the strobilus, the effect being to push the sporophyll-bearing portion beyond the scales (fig. 9). A similar elongation of this region is reported for *Torreya californica* (20). COULTER and LAND described an enlarged pith region in the axis of the strobilus in *T. taxifolia*, which the authors suggest may be "an important storage region for the strobilus." No such enlarged region was found in *T. canadensis*. In addition to the elongation of this portion of the strobilus axis there is also an elongation of the stalk of the sporophyll (cf. figs. 9 and 10), resulting in the separation of the sporophylls from one another.

The sporangia do not hang freely from the underside of the disk, but are fused with the stalk on the inner side (fig. 9), and laterally are separated from one another only by thin partitions, the external furrows between the sporangia not extending all the way to the center, the sporophyll and sporangia thus constituting a very compact structure. RICHARD (19), STRASBURGER (22), and GOEBEL (10) gave accounts of the dehiscence of the sporangium of *T. baccata*, in which they pointed out the rupture of the sporangia at the base and the umbrella-like movement of the epidermal wall.

The process is the same in *T. canadensis*, the breaking of the thin-walled epidermal cells at the base of the sporangium in a circle around the base of the stalk, the rupture of some of the cells at the side of the sporangium, and the hygroscopic rôle of the thickened epidermal cells resulting in the wall of the sporangia spreading out in umbrella form, the thin partitions between the several sporangia also being broken in the process.

When young the strobili rudiments are erect in the axils of the leaves, but as they develop they become oriented in such a way as to hang pendent on the lower side of the shoot, the fertile portion of the strobilus being directed downward. GOEBEL (10) regards the position and the method of dehiscence such as to secure the most advantageous distribution of the pollen.

Vascular features

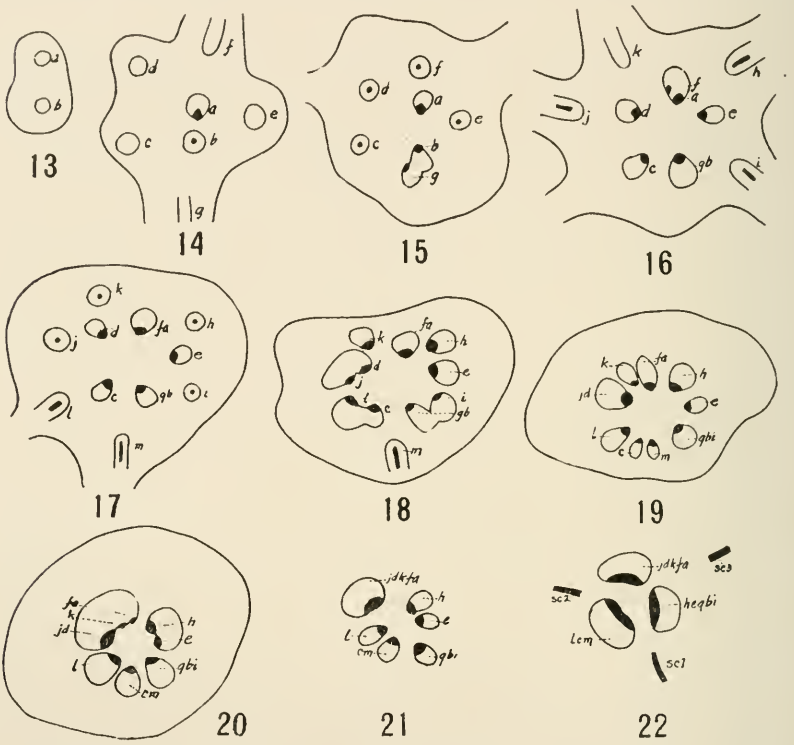
Since the reproductive organs, and especially the staminate structures, are regarded as among the most conservative of plant organs, a consideration of the vascular anatomy of the staminate strobilus is not without interest. While the ovulate strobili of conifers have been the subject of considerable investigation and discussion, in their vascular as well as in other features, the staminate strobili have not received much attention in their vascular anatomy, probably not as much as they deserve in view of the conservative nature generally assigned to them on other grounds. The only reference to this feature of *Taxus* is by STRASBURGER (22), who gave the arrangement of the scales of *T. baccata* and states that each stamen contains a bundle which passes into the stalk.

Like any other branch, the strobilus axis receives 2 bundles from the cylinder of the leafy shoot. These are semicircular in outline, and by meeting at their edges soon form a closed cylinder, broken here and there by the gaps formed by the weak bundle traces of the scales. In the lower portion of the strobilus, where the scales are small and decussate, the small traces often end in the cortex and do not reach the scale itself. The traces for the upper scales are better developed and extend for some distance into the midrib of the scale, especially in the 2 or 3 uppermost scales. Although the axis cylinder, as well as the cortical portion of the scale traces, are

collateral endarch, in their terminal portions they contain not only centripetal xylem, but are also accompanied by transfusion tissue which may be both dorsal and lateral to the xylem elements (fig. 38).

At the level of the uppermost scales the cylinder consists of 3 or 4 large bundles (figs. 22, 48) which extend into the fertile portion of the strobilus, where they branch, giving off finally a branch to each sporophyll, the bundle extending a little way into the base of the sporophyll stalk. In a young strobilus these bundles are represented only by elongated thin-walled elements, evidently procambium strands, which traverse the region between the base of the sporophylls and the level of the upper scales (figs. 7, 8). These strands remain in this embryonic condition until near maturity, when they elongate and take on their vascular features in connection with the growth of the "elongating region" of the strobilus axis. In this "elongating region" the pith becomes larger in diameter than in the lower portion of the strobilus, but shows no evidence of being in any way a storage region; in fact, there would be little use of a storage tissue at this stage in the development of the strobilus. The several large bundles of the strobilus axis extend for some distance into the "elongating region" and then give off branches to the various sporophylls, each of the large bundles supplying several sporophylls in this way (figs. 13–22). Some of the branches may unite and then separate (see the behavior of bundles *h* and *c*, and also of *l*, *c*, and *m*, in figs. 18–22), although usually the bundles pass rather directly to the base of the sporophyll (figs. 14–17, 44–47). Throughout the entire axis there is relatively a stronger development of the phloem than of the xylem, the latter forming a narrower zone than the former (fig. 48). Both xylem and phloem reach their greatest development near the level of the upper scales (figs. 9, 22), above this the xylem forming only a very narrow portion of the bundle. Throughout the strobilus the xylem consists of spirally thickened tracheids with bordered pits, the tracheids being rather short, however, although in the elongated region of the axis they are somewhat longer than at a lower level and the bordered pits are fewer in number. The phloem of the portion above the scales shows very little of the pitting present at a lower level, consisting

of elongated cells similar to those of the younger condition of the strobilus. Occasionally the xylem extends a short distance into the stalk of the sporophyll, the bundle here, however, usually consisting



FIGS. 13–22.—Cross-sections of mature strobilus at approximately levels indicated by numbers to left of strobilus shown in fig. 9; branches of bundles to various sporophylls indicated by *a*, *b*, *c*, etc., xylem indicated by black; bundles *a* and *b* supply terminal sporophylls; union of bundles indicated by combining letters, as *jd* in figs. 17–19; fig. 19 shows complete cylinder below lowermost sporophyll; in fig. 18 *l* and *c* united, in fig. 19 separated, in fig. 20 *c* and *m* united, and in fig. 22 *lcm* one of the 3 large strands from sterile portion of axis; fig. 22 also shows traces to 3 uppermost scales, *sc1*, *sc2*, and *sc3;* note concentric character of terminal portions, as in *c*, *d*, and *e*, in fig. 15; ×36.

only of the phloem portion (fig. 39), the xylem usually ending within the cortex of the axis.

The bundles of this region are collateral endarch in the lower portions. In the upper portions, however, the bundles frequently show centripetal xylem (figs. 40, 44), giving mesarch bundles, and

in some cases the smaller xylem elements are on the outside of the bundle, indicating a possibility of exarch structure (fig. 42). In addition, the xylem elements, in the terminal portions of the bundles, become more and more placed toward the center of the bundle, giving virtually a concentric bundle of a few xylem cells surrounded by the phloem portion of the bundle (fig. 41). No transfusion tissue was found elsewhere than in the scales.

Discussion

Perhaps the two most important features of the staminate strobilus of *Taxus* are the peltate sporophylls and the character of the vascular bundles of the scale and sporophylls. The peltate (epaulet) type of stamen occurred among the Paleozoic Cycadofilicales, in the *Crossotheca* forms, but the sporangia were bilocular and dehisced by a longitudinal slit along the adaxial face, the bilocular character being different from that of the modern gymnosperms. Peltate stamens are not known in Bennettitales, and none occur in the Cycadales. The peltate stamen has been carried forward to modern plants through the Cordaitalean line, in all probability, although so far as is known the stamens in the Cordaitales bore terminal erect sporangia. As COULTER and CHAMBERLAIN state, however, "it cannot be supposed that the stamens of so great a group were uniform in type," and it is very possible that peltate stamens occurred there also. The sporophyll of *Ginkgo* gives a suggestion of the peltate type of stamen, in occasionally having more than 2 sporangia, in the regular occurrence of more than 2 sporangia in fossil forms, and in the possibility, pointed out by Miss STARR, that the mucilage cavity replaces abortive sporangia. Among Coniferales there is a suggestion of the peltate stamen in the Araucarineae, and stamens of true peltate form occur in such forms as *Widdringtonia*, *Torreya*, and *Taxus*. In *Torreya* the true peltate character is generally obscured in the adult sporophyll owing to the development of the resin cavity from 3 of the 7 sporangium beginnings. Hence it is seen that peltate stamens, in one form or another, are scattered from Cycadofilicales to modern conifers, and there is no necessity of regarding such a sporophyll as that of *Taxus* as being of recent evolution. Assuming peltate

sporophylls in Cordaitales as probable, their continuation in Ginkgoales and Coniferales is quite possible, abortion of some of the sporangia in the formation of mucilage or resin cavities, in such forms as *Ginkgo* and *Torreya*, obscuring their true nature, but showing the true peltate character when all of the sporangia develop, as in *Taxus*. WORSDELL, following the view put forward by CELAKOVSKY (2), considers the peltate sporophyll of *Taxus* to have been derived from such a form as occurs in the Cordaitales, where the pollen sacs are "erect and terminal on the radial sporophyll," through such forms as found in *Cephalotaxus* and *Torreya*, where the pollen sacs are "sub-terminal and pendulous, owing to a slight prolongation of the axis of the sporophyll, between and beyond the sacs, in a small protuberance," this condition being intermediate between the Cordaitales situation and *Taxus*, "where the extended terminal portion has become enlarged and flattened out into a very distinct peltate structure." "*Taxus* thus represents an advance from the earlier types of *Cephalotaxus*, *Ginkgo*, etc., toward the subpeltate dorsiventral type of sporophyll of the true Coniferae." One must question the necessity of such an explanation for either the peltate sporophyll of the taxads or the dorsiventral one of most conifers, in view of the historical occurrence of both of these types in forms more primitive than even the Cordaitales.

The significant features of the vascular anatomy of the strobilus are the mesarch character of the terminal portion of the scale bundles, as well as the appearance of centripetal xylem in the terminal portion of the sporophyll bundle, where the bundle is not only mesarch at times, but may also be exarch and concentric. This indicates the very conservative nature of the staminate strobilus. These primitive features, however, occur only in the terminal portions of the strobilus, which may be regarded as an argument in favor of the "advanced" character of *Taxus*, compared with forms with more abundant centripetal xylem.

Summary

1. The staminate strobili occur in the axils of the leaves. The buds can first be distinguished from other types of buds by the broad apex.

2. The sporophyll primordia first appear as slightly rounded lobes above the general surface and may arise in acropetal succession.

3. The archesporial initials are hypodermal cells and develop according to the eusporangiate method. There are 4–8 of them, distributed around the margin of the primordium.

4. The sporogenous tissue reaches the mother cell stage about October 1, and forms microspores about 2 weeks later. There is no abortion of sporangia such as occurs in *Torreya*, the sporangia occurring in a circle around the stalk of the sporophyll.

5. The sporangium wall is usually 2-layered. The tapetum arises from the peripheral layer of the sporogenous tissue and persists until after megaspore formation.

6. The epidermis of the sporangium remains alive and thin-walled at the base, dehiscence being accomplished by the rupture of these cells at maturity, by the elongation of the stalk of the sporophyll. Owing to the disintegration of the sporangium wall, the epidermis is the functional wall in the later stages.

7. The strobilus matures the latter part of April. Just before maturity there is an enlargement and elongation of the axis, pushing the sporophylls beyond the scales.

8. The strobili of *Taxus canadensis* are somewhat smaller than those of *T. baccata*.

9. The strobilus bundles are collateral endarch, excepting in the terminal portions of the scale bundles and the sporophyll bundles, where they may be mesarch, and in the latter show indications of occasional exarch structure, the terminal portion of these bundles also being concentric.

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EXPLANATION OF PLATES XXIV–XXVI.

All drawings were made with a camera lucida; text figs. 1–10 and 13–22 are drawn to the same scale, with a magnification in reproduction of approximately 36; text figs. 11–12 and all plate figs. are drawn to the same scale, reduced one-half in reproduction, having a magnification of approximately 475.

PLATE XXIV

FIG. 23.—Young sporophyll primordium, showing 2 archesporial initials in hypodermal layer of rounded primordium.

FIG. 24.—Archesporial initial in hypoderm; division of epidermal cell.

FIG. 25.—Archesporial initial in tangential section.

FIG. 26.—Archesporium of 2 cells.

FIG. 27.—Metaphase in division of archesporial initial.

FIG. 28.—Late stage in division of archesporial initial.

FIG. 29.—Primary wall cell (outer cell) and primary sporogenous cell (inner cell), resulting from division of initial.

FIG. 30.—Primary wall cell and division of primary sporogenous cell.

FIG. 31.—Primary wall cell has formed 2 tiers of wall cells, in one of which division is taking place; primary sporogenous cell has divided anticlinally, forming 2 sporogenous cells.

FIG. 32.—Lobe of young sporophyll, showing small mass of sporogenous tissue, 2-layered sporangium wall formed on outer side, and meristematic basal portion of epidermis differentiated from remainder of epidermis.

FIG. 33.—Somewhat older stage than fig. 32.

FIG. 34.—Sporangium wall complete, entirely surrounding sporogenous mass; latter part of July.

FIG. 35.—Older sporangium, showing differentiation of tapetum from sporogenous tissue.

FIG. 36.—Portion of sporophyll, showing epidermis, 2-layered sporangium wall with narrow flat cells, tapetum (1 cell binucleate), and microspores; winter condition.

PLATE XXV

FIG. 37.—Transverse section of portion of lower scale, showing stoma on inner surface and heavily cutinized epidermal walls, especially on outer surface.

FIG. 38.—Transverse section of portion of upper scale, showing vascular bundle and inner epidermis of scale; in vascular bundle note centripetal xylem and 2 transfusion cells, 1 dorsal, 1 lateral to xylem.

FIG. 39.—Transverse section of bundle *a* of fig. 13, showing phloem character of bundle; no xylem present.

FIG. 40.—Bundle *a* at level of fig. 15, showing mesarch character.

FIG. 41.—Bundle *e* of fig. 15; single xylem cell surrounded by phloem.

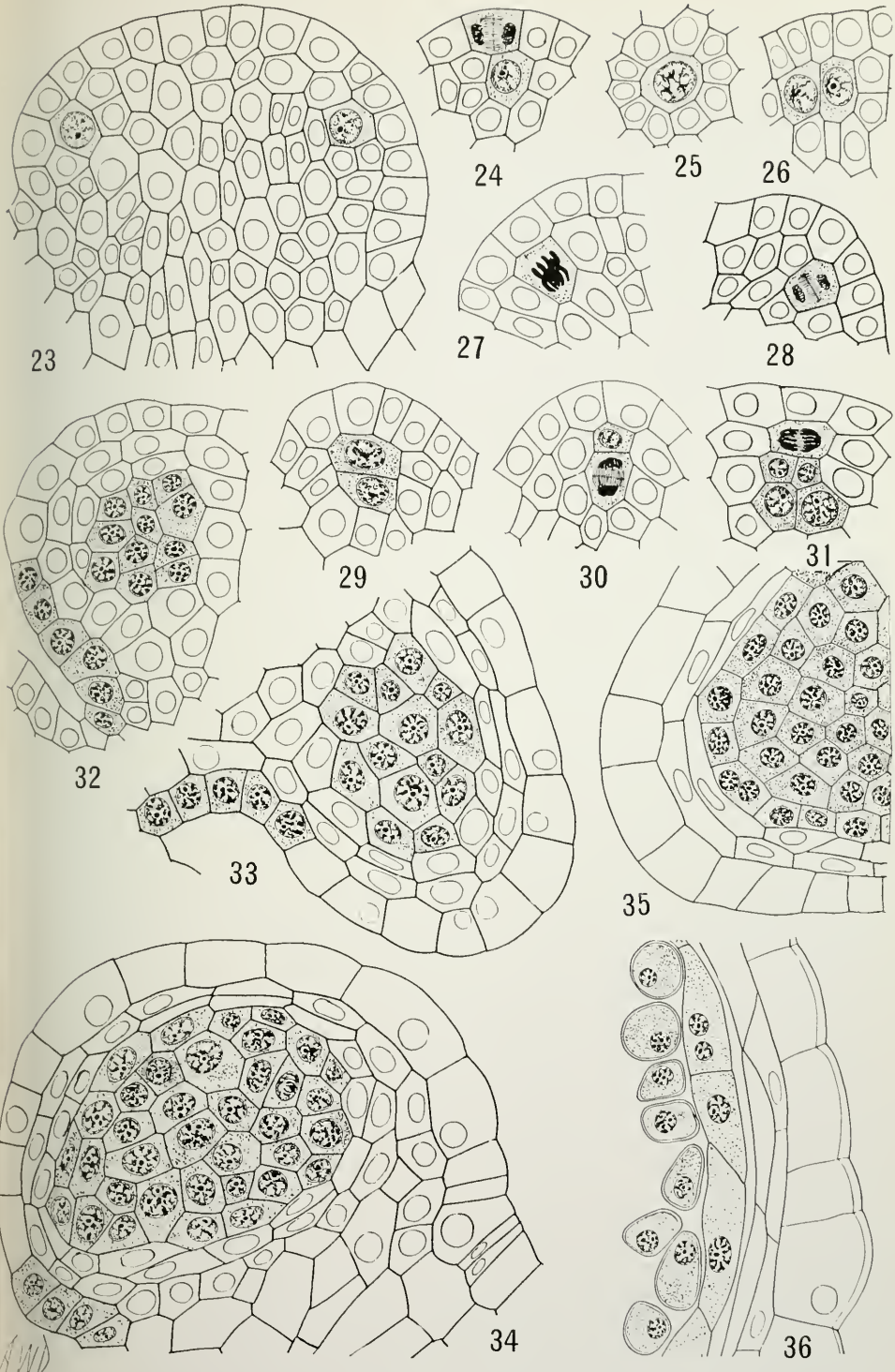
FIG. 42.—Bundle *e* at a lower level; large xylem cell centripetal to smaller ones, indicating possible exarch condition.

FIG. 43.—Bundle *gb* at level of fig. 17, showing collateral endarch character

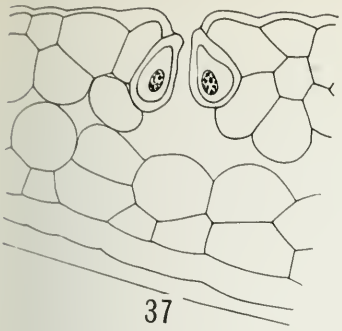
PLATE XXVI

FIGS. 44–47.—Fusion of bundles *f* and *a* (see figs. 15–17): in fig. 44 *f* is concentric, *a*, mesarch collateral; section 60 μ below level of fig. 15; fig. 45 enlarged view of bundle *fa* of fig. 16, 80 μ below fig. 44; fig. 46, 2 bundles near together 20 μ below fig. 45; fig. 47, fusion bundle *fa* 30 μ below fig. 46.

FIG. 48.—Transverse section of bundle *lcm* near level of fig. 22.



DUPLER on TAXUS



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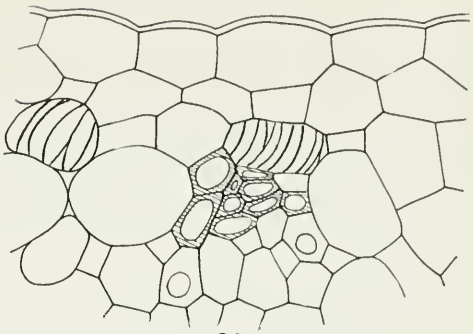

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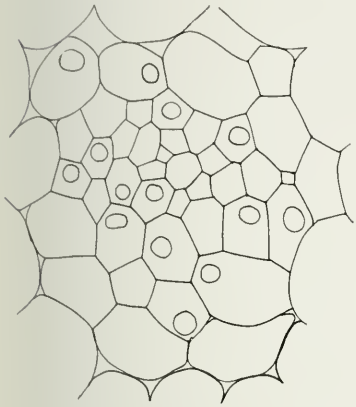

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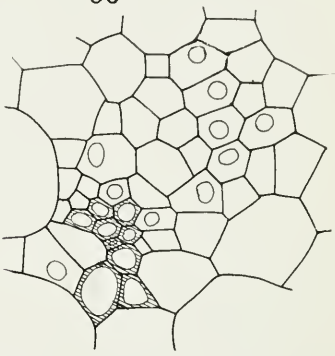

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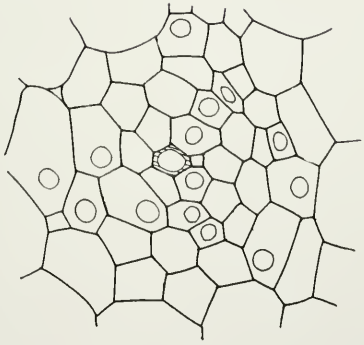

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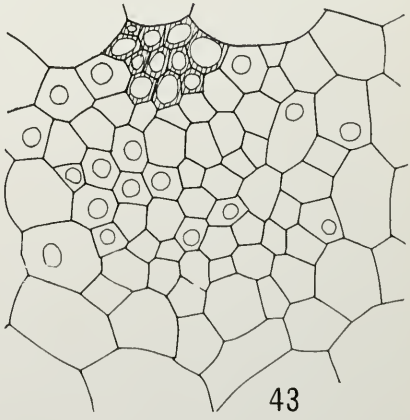

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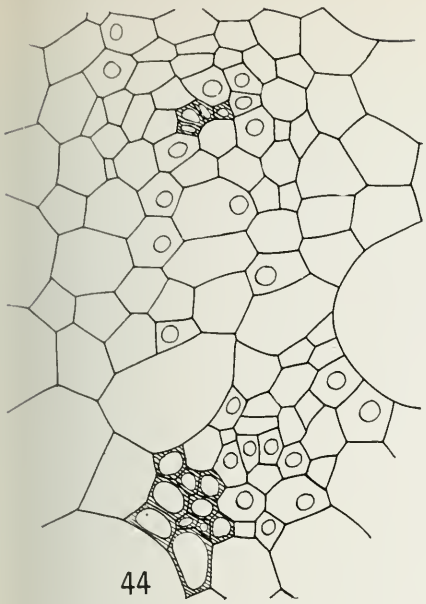
DUPLER on TAXUS



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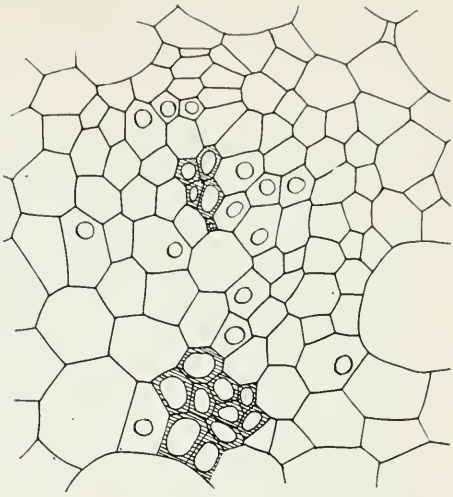

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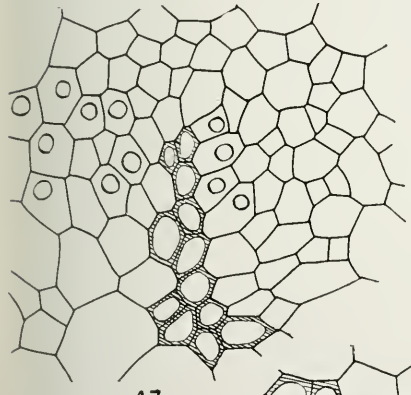

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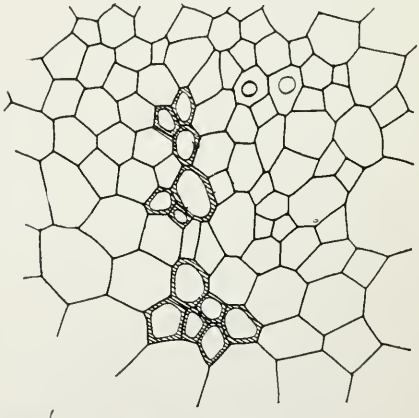

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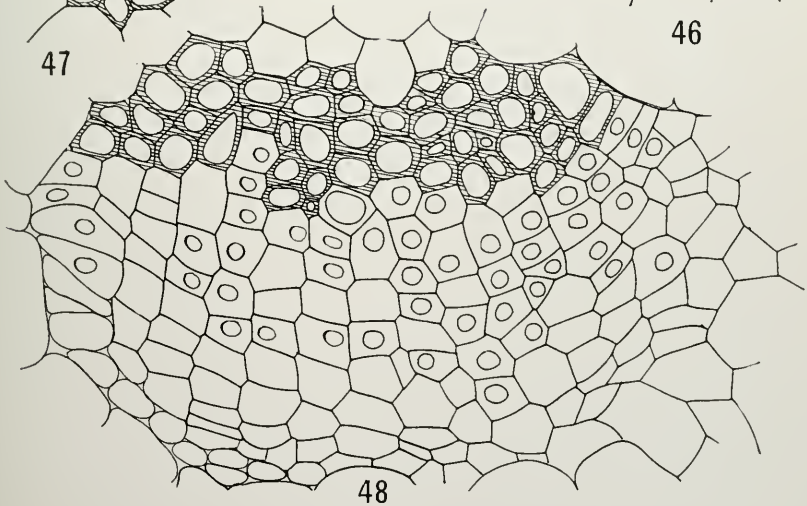

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A.W.D.

DUPLER on TAXUS

UPLAND SOCIETIES OF PETOSKEY-WALLOON LAKE REGION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 256

(WITH ONE FIGURE)

H. D. CLAYBERG

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UPLAND SOCIETIES OF PETOSKEY–WALLOON LAKE REGION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 256

H. D. CLAYBERG

(WITH ONE FIGURE)

Introduction

The writer became familiar with this region through spending the summers there for the past 12 years. During this time the rapid destruction of the few wild areas left suggested that studies be made while natural remnants were still available. The observations on which this paper is based have been carried on for at least three years. Besides the general data, 40 quadrats were made and a map of the plant geography (to be published later) was drawn.

The topography of this area was largely determined during the Pleistocene and Postglacial. LEVERETT and TAYLOR (16, 17) have covered this phase ably. At the time of the formation of Lake Chicago beaches this region was ice-bound, later forming part of the submersed area which gradually emerged as the waters changed from Lake Algonquin to the Nipissing Great Lakes, and through the Post-Nipissing stages to end in Lake Michigan. This periodic subsidence left the Algonquin, Nipissing, and later beaches (together with scattered morainal lakes inland), but erosion here has eaten away much of the Post-Nipissing levels.

The region at present is underlaid with Devonian deposits. Inland the surface layer is Upper Devonian, being largely black Antrim shale; while a marginal strip of about 3 km., from Petoskey west, and all territory north of the south margin of the Inland Route are covered with Middle Devonian. The latter contains the Petoskey limestone, which outcrops along the shore of Little Traverse Bay, either as shelving bedrock or limestone cliffs, the beds dipping inland. The lakes and channels have a layer of subaqueously deposited sand, covered in most places by black muck (fig. 1).

The region studied lies in Emmet and Charlevoix counties, Michigan. It includes a strip about 2 km. wide along Little Traverse Bay from Bay Shore to Idylwilde, together with Walloon

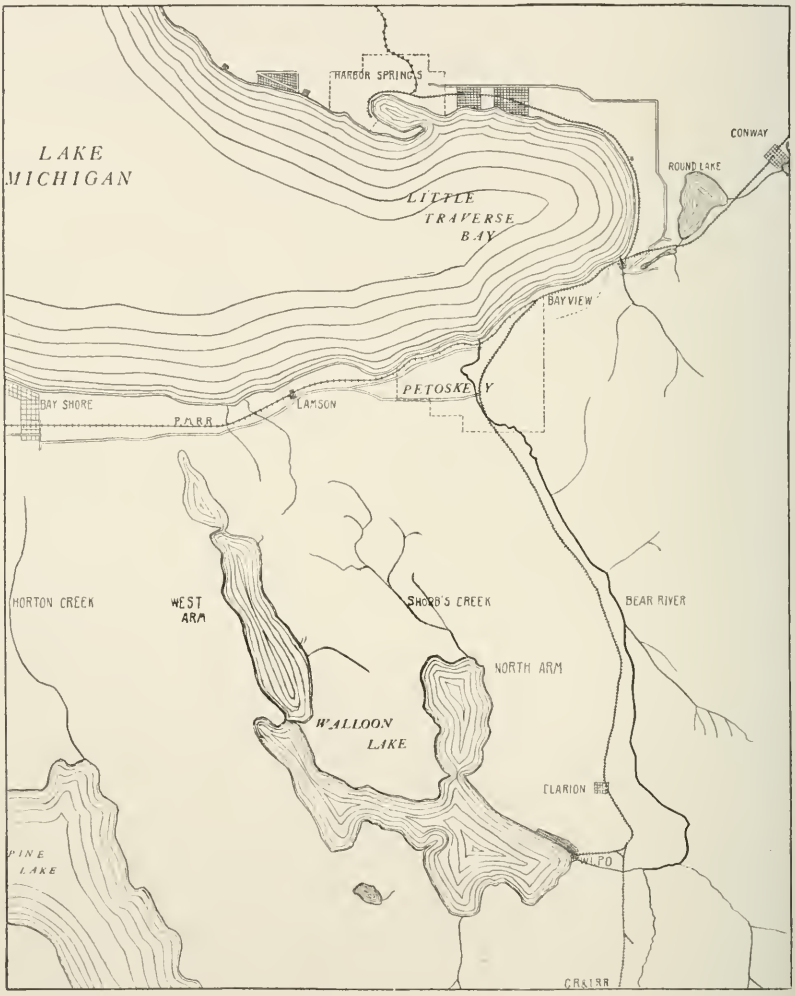

FIG. 1

Lake and surrounding shores. Bear Creek and Resort townships in Emmet County are also included.

The area of the region exceeds 260 sq. km. (about 100 sq. mi., of which about four remain in forest) and includes four upland

masses, one between Pine and Walloon lakes, another between Bear Valley and Walloon Lake, a third between the latter and the Inland Route, and a fourth north of the Inland Route. Inland Route is a valley extending from Kegomic to Cheboygan, with a continuous water channel running through it, beginning with Round Lake on Little Traverse Bay and emptying through Cheboygan River. While the only river of any size is Bear River (flowing through a broad and deep valley northward to Petoskey), many small creeks empty into the lakes and into Lake Michigan.

The topography is irregular and hilly, the upland being mostly highest behind the Algonquin bluff, sloping gradually down to the southeast in the territory south of the Inland Route, and to the northeast in the portion north of the same. The varied glaciated topography suggests wide floristic diversity, combined with youthful form (6), and this is the actual state found. The region was entirely covered with forest before settlement; the uplands with the climax maple-beech forest of the region, and the channels like the Bear Valley, together with the swamps and creek valleys, being mostly a continuous stand of *Thuja occidentalis* L.

The classification of COWLES (5, 6) has been found more suitable in analyzing the formations of the region, but this may partly be due to the fact that less soil diversity was observed than found by WHITFORD (25) along Lake Superior, so that an edaphic arrangement seemed less desirable. The edaphic factor, however, could not be excluded, as in cases where one type of soil alone was studied (9, p. 46). COONS divides his successions into swamp (lagoon→forest) and sand (beach→pine barren) series (20, p. 60).

Upland types preceding climax

The upland societies remaining include only the late tree stages, the earlier ones being lost. For convenience of treatment similar areas in the older parts of other series will be discussed here. Three apparent stages are seen.

PINE FOREST

Only *Pinus Strobus* L. and *P. resinosa* Ait. occur. *P. Banksiana* Lam. has not been found, although it occurs around the south end

of Lake Michigan, and in the pine barrens of northern Michigan as near as Wolverine in Cheboygan County. In general, the pine occurs in three places: (1) on the high hills back of Walloon Lake, (2) on Algonquin and Nipissing bluffs, and (3) as an early stage in dune forest succession.

The first location is a xerophytic open society of red pine sloping southward to the lake. The herbage below is dominated by ericads such as *Gaultheria procumbens* L. and *Vaccinium vacillans* Kalm. Occasional artificial clearings show an apparently succeeding stage whose components are crowded and mainly of shrub size. Here *Cornus* (*Baileyi ?*) and *Viburnum acerifolium* L. dominate. Following this is an obviously secondary society (may be absent in the primary series), taller than the preceding one and primarily *Betula alba* L. var. *papyrifera* Spach., with a mixture of *Populus grandidentata* Michx. and *P. tremuloides* Michx. Oak seems to follow.

The second type, almost entirely white pine, shows the oldest pines seen, growing on slopes approaching 45°, with sparse vegetation below characterized by *Solidago racemosa* Greene and *Shepherdia canadensis* Nutt. The xerophytic conditions here obtaining are indicated by leaves of *Aralia nudicaulis* L. 12 cm. across and 10 cm. tall, as well as by beds of *Polytrichum commune* L. Where cleared, the succeeding thickets are white birch with some *Prunus pennsylvanica* L. f. and *Amelanchier*.

The third type is a mixture of the two species, with white pine dominating, but with other conifers present. Among the particularly characteristic undershrubs occurring are *Corylus rostrata* Ait. and *Rosa acicularis* Lindl., while the herbage is largely of the ericoid type. At Menonaqua the full series is seen, but north of Harbor Springs erosion has eaten back into the pine society; the xerophytic conditions resulting permit persistence of much of the dune flora (telescoped succession).

As at present limited, pine occurs here near water in positions exposed to direct wind and of noticeably xerophytic nature. This agrees with its probable status as a relict tree formerly covering the upland. Transeau (24) believes conifers reached their present distribution in the lower peninsula of Michigan by way of the lake shores.

Oak forest

Quercus rubra L. furnishes an unimportant and rare type. Stands are seen near Walloon Lake, and on the Algonquin bluff north of Harbor Springs, which extend inland in places for some distance. This tree occupies the same sort of habitat as the pine, and probably succeeds the latter in certain areas. Oak also covers Harbor Point, a low Post-Nipissing area. The discontinuous distribution shown suggests relatively recent seeding at Walloon Lake. Along the bluff north of Harbor Springs oak succeeds pine, when trees of the former are near and the pines are far enough apart (or have been cut or burned off). This occurs especially where the slope is not steep. Invasion of the adjacent upland by oak has occurred in one place (5). *Quercus velutina* is absent from this region (13).

Hemlock forest

The few stands of *Tsuga canadensis* Carr. left are confined to areas similar to those bearing pine, but of less xerophytic nature. It appears that any area bearing hemlock in this region is ecologically prepared for the climax forest, for, aside from the fact that hemlock is more or less common in the climax forest itself, and that hemlock stands normally bear some deciduous trees, the undergrowth and seedlings of an open hemlock forest are usually deciduous, and where the trees are cut off the young growth is largely maple and beech. The periodic reproduction of conifers may have a disadvantageous influence on their persistence. On the low hills bordering Walloon Lake a nearly pure stand is common, running from an average of 20 cm. diameter to a maximum of 80 cm. In such a primary society few herbs or seedlings are scattered over the brown needle layer. The characteristic plants are *Taxus canadensis* Marsh, *Lycopodium lucidulum* Michx., *L. clavatum* L., *Clintonia borealis* Raf., and *Mitchella repens* L. Where cut off, the sapling flora is almost exclusively deciduous, being about 60 per cent *Acer saccharum* Marsh, mixed with *Fagus grandifolia* Ehr., *Acer pennsylvanicum* L., and *A. spicatum* Lam.

Beyond Menonaqua the pines adjoin a hemlock beech society, which very likely will succeed them. This represents the richest

hemlock type seen, probably because farthest from the shore and most sheltered from the wind. The presence of many balsam and some oak seedlings, and the absence of sugar maple, make the next stage uncertain. Dense thickets of *Corylus rostrata* Ait. and much *Taxus* are characteristic. The hemlock on a Post-Nipissing level west of Harbor Springs is similar, but is mixed with *Abies balsamea* Mill. and *Thuja occidentalis* L. The Algonquin cliff west of Petoskey in several places bears large hemlock stumps of uniform (71–75 cm.) diameter, indicating that it was once largely occupied by a fine hemlock forest. The trees were cut sometime ago, for the secondary forest is nearly grown (average diameter 25 cm.), being beech, sugar maple, and *Betula lutea* Michx. f. A constant associate on open banks and cliffs is *Polytrichum commune* L., taking here as prominent a place as *Taxus canadensis* does in the level and denser part of the forest.

Climax forest

Seriation

The composition of the climax primary forest of the region has long been considered constant from the time the maple and beech reach dominance and respectable age. This is true floristically, but not ecologically or physiologically; for a climax formation is static in species, but dynamic as to individuals. Analysis of sufficient territory shows the forest to be more or less of a patchwork composed of trees in varying stages of development.

Cooper (4) found the climax forest he studied to be a "complex of windfall areas of differing ages, the youngest made up of dense clumps of small trees, and the oldest containing a few mature trees with little or no young growth beneath, those of a single group being approximately even-aged. This mosaic or patchwork changes kaleidoscopically through long time spaces, but the forest as a whole remains the same, changes in various parts balancing each other." His studies were of a coniferous forest. The climax here is deciduous, so differences are to be expected. The forest floor is lighter and the next generation starts sooner in the case of the maple-beech forest. The patches observed in the climax forest of this region are too large to consider as the result of one tree fall. Further, they

would all have to approach the oblong or elongate form, whereas they are irregular where discernible, for the maple-beech forest is not to be considered as either patches of cleanly distinct even-aged trees, or as continuous forest with each generation even-aged throughout. It rather varies between these two ideals as limits.

Since the seriation is of individuals, the climax is not final, but recurrent, and during the development of each rough area or patch certain ages are to be recognized, each with fairly definite form, height, and spacing. At any one locality they follow each other in regular order, two or more commonly superposed, and adjacent areas independent of each other.

Definition of these ages is attempted approximately as follows:

	Age	Average diameter	Average spacing	Average height	No. per 100 sq.m.
Seedling.....	1	5 mm.	40 cm.	40 cm.	670
Sapling.....	2	2 cm.	65 cm.	4 m.	300
Young adult....	3	15 cm.	3 m.	10 m.	10
Adult.....	4	50 cm.	6 m.	30 m.	3
Old tree.....	5	65–85 cm.	8–20 m.	35–40 m.	1

ECOLOGICAL LIFE HISTORY.—The flowers and fruits of the climax forest are mostly inconspicuous. Undeveloped fertile seeds are always present, as is shown by the abundant germination in clearings. The latter also emphasizes light as a critical factor.

Since the forest determines the intensity, amount, and continuity of the light penetrating, the number of seedlings (age 1) and their distribution depend largely on the forest's age. Many seedlings die, but are easily replaced They seem rare, but in reality often average 7 per sq. m., forming a scattered layer 20–60 cm. in height. The typical seedling form shows a slender, often branched, stem. The leaves are loosely corymbed or in one or two horizontal layers. The oval foliage outline results from free lateral growth (perhaps also spread to catch maximum of light). Apparently most of them remain nearly stationary for years. The taller ones appear distorted and dying, as if starved for light, which seems to decrease approaching the base of the sapling foliage.

Removal of the old trees above (15) permits freer elongation of the saplings. The seedling stratum becomes better lighted and watered, due to recession of foliage above and roots below. More

seedlings germinate to fill the gaps, and elongation results in the formation of a new sapling stand (age 2) as the trees above reach age 4. The sapling axis is long and straight, forks and side branches equaling the stem are rare, and the foliate part of the tree, although polygonal in cross-section, approaches a right cylinder. The lowest branches are dead twigs, the later ones are horizontal or angle up.

A fine close sapling stand is the culmination in percentage of volume occupied. As the size of a stand increases, the distances between its trees increase also, and it is believed that a law will here be found to control relation of diameter and spacing of trees. The sapling age shows maximum increase in size for given decrease in number per unit area, hence competition between trees of equal age is keenest here.

With removal of another generation the saplings elongate, but intensity of vertical growth decreases, for the relatively open spacing permits lateral growth and reapproach to the typical broad form shown by isolated trees in field and pasture. In passing from the second to the third age a transition in branch form is seen, from the filiform type of evanescent branch to the massive type of permanent branch characteristic of the adult. These originate far above the sapling tops and hence are developed later. Comparison of the young adult and sapling stages with regard to ratio of height to breadth suggests partial etiolation in the latter. All saplings with forked axes are eliminated, since no adults are seen with forks at sapling level. Naturally a biaxial shoot is at a disadvantage under active competition with those supporting but one.

With further thinning of population the adult stage (age 4) is reached. This is the true ecological climax. The maximum foliage display and culmination of vitality are seen here. A typical tree was studied, felled, and measured. There was no sign of lost branches or decay, all branches bearing a rich display of leaves in normal position. The trunk was clean, straight, and subcylindric, with the lowest branch 25.3 m. from the ground. The diameter basally was 53 cm. and the tree was 32.5 m. tall. The crown was oval, with 12 major branches. The duramen showed a central cavity 8 cm. wide at the base, with its cone point ending about 2 m. above

ground. Because of this cavity the age could only be estimated by proportion; the tree was approximately 250 years old (allowing for thicker early rings).

The senile or last stage (age 5) is scattered, because definite spacing is lost. Many primary limbs are gone, adventitious branches along the trunk and on otherwise dead limbs and stubs taking up the work. The heartwood is largely rotted. The sawed-off stump of one very old tree showed a cross-diameter of 120 cm., but only a margin of 15 cm. around the outside was wood, the rest being hollow. The base, at or near ground level, is often inhabited by a colony of big ants, and the breaking point is normally at this place. A certain degree of pliability is still retained in ages 4 and 5. The latter are apt to sway widely in a wind, some creaking loudly also under the strain; yet the tree may stay thus at the verge of fall for years.

Approach of death is equally indicated by the crown where symmetry is lost by branch fall. The top of an old tree is always ragged. These trees attain the maximum of height and diameter. They represent a wider range of age, dimensions, and form than any other of the life stages, partly because of their liberty of freer development than the younger trees below.

The beech follows the maple in general, but it is stockier, broader, and shorter, reaching each age much more quickly. Its terminal bud is weaker, and the tree apex is often injured by falling trees, lightning, and other destructive agents, so that the nutrients go to several branches near the top. As a result it is strikingly deliquescent and rarely develops a bole over 15 m. in height below the branches.

Stratification

Maximum complexity.—Investigators in the tropics have noted 5–7 strata in the rain forest (21). These were primarily due to the leafing out of the various tree species at different levels. It has been assumed that little or no stratification occurred in the climax maple-beech forest, the belief being partly based on the poverty of tree species (but two or three important) and the far lower degree of luxuriance as compared with the tropical rain forest.

Lower forest	Soil stratum: here lie roots, youngest farthest up.
	Leaf stratum: thin crisp continuous layer.
	Herbage stratum: includes seedlings also (age 1).
Middle forest	Sapling trunks: first really open stratum; shrubs here.
	Death stratum: layer of dead twigs below sapling foliage.
	Sapling synfolium: sapling foliage layer.
Upper forest	Tree trunk stratum: ample light first reached.
	Upper synfolium: broken zone of adult tree foliage.

The strata of any one generation are best shown and fullest developed at the sapling age. They are not so well formed in the seedling and are breaking down in ages 3 to 5. Only major layers are listed. For this reason the seedling synfolium is not accorded separate rank (although thicker than leaf stratum).

Synfolium.—The synfolium is the layer formed by leaves of trees of the same age. It is the result of photosynthetic need in crowded sessile individuals. It must be dealt with not only as compound, with the unit the foliage leaf, but also as a mass. The placing together of all the synthetic tissue of a group of trees is of serious ecological importance. The leaf placing, together with the crowding of the trees, makes the vertical section of an individual show a nearly rectangular foliage mass. The synfolium governs its depth by means of the light relation. It also controls the amount and composition of the herbage below. In the general discussion here given, the synfolium of the sapling is taken as type.

While the synfolium continually and gradually ascends as the trees grow (no sudden jumps), the history of the foliage layer shows characteristic stages. Since the seedlings are scattered, their foliage layer is discontinuous horizontally. It is very close to earth level and is but 20–40 cm. vertically. As the sapling age approaches, the small foliage masses fuse into a continuous layer, having a much greater vertical section, and both upper boundaries parallel, horizontal, and nearly flat. This is the ecologic climax of the synfolium; here it reaches its greatest definition and density. Most of the growth is strictly limited to the top at this age, but later ages show the maple in its true light as more typically a deliquescent tree.

At the sapling age the synfoliar depth (from its top to its bottom) is 3–4 m. As it recedes from the ground its upper surface becomes

uneven and covered with the free cones of the young adults, while spaces creep up from below. These result because lateral growth is insufficient to maintain closure. Increased lateral spacing now permits increased lateral growth, one of the prime factors slowing vertical elongation. Approaching the adult stage (age 4) the layer breaks up into its component tree masses. This occurs by rifting (vertical or horizontal breaks due to tree or branch fall), the gaps becoming nearly unfillable at age 3, for closure is either by elongation of a younger tree or by lateral growth of the adjacent tree circle. This age is the first one free vertically and laterally.

A further step is the breaking up of a tree unit into foliage clumps, one or several to a branch. Finally, many of the oldest lose all primary foliage, the trunk and branches bearing scattered handfuls of leaves. This secondary foliage is borne on slender twigs. developed from adventitious buds. Gradual fall of the last age destroys all semblance of a foliage stratum.

Recession occurs in two main ways (trunk elongation unimportant): by shedding of leaves and branches at the synfolium base (the synfolium is self-pruning during the growing season), and by apical growth, the stems adding new leaves and branches, thus extending the synfolium compass vertically. With increase of synfoliar distance (from ground) and rifting, the herbage layer receives increasingly stronger light; thus the tree seedlings are stimulated to more active growth and the illumination of the forest floor decreases again.

The sapling synfolium contrasts with the trunk strata above and below, in apparent space occupied, color, and opacity. The lighting of the trunk stratum above is much greater, and that of the dead branch layer much less, being composed of flat, thin, horizontal tissue plates. The synfolium seems to have the ideal structure and arrangement for maximum of surface, light absorption, synthetic efficiency, and carbon dioxide use, together with the minimum material, volume occupation, and transpiration. The apparent effect on the eye gives impressive display and exaggerated idea of solidly filled space. This effect is heightened on passing from the bright sunlight into the dense shade of the forest.

YAPP (26) makes some interesting observations on evaporation at different levels in an English marsh, and SHERFF (22) on an

American marsh, finding evaporation rate proportional to height above the soil. These suggest that data on the levels of the climax forest of this region would be significant. GATES (8) compares evaporation at the chamaephytic layer in different societies but not at different levels. He believes evaporation a result, not a cause, of succession.

ENVIRONMENT

Competition is affected by several influences: physical and chemical factors, parasites, and individuals of the same or an older generation. Scattered among the herbage are tree seedlings, many of them dead or dying. In fact the younger the group, the more die. No competition between seedlings occurs except as two are found within short radius of each other. The critical competition for them occurs with the older trees in the form of light interception (most important) from above and nutrient interception from below. Since the lifting of the light inhibition is very slow in terms of potential seedling growth, the plasticity of seedlings becomes a factor. Being so adaptable, one can fit itself to any rift by lateral growth; occasionally one with over 90 per cent of its leaves on a far side branch will be found. Maximum spatial crowding is reached in the sapling age, and consequently the most critical competition of the life cycle occurs here.

Approaching the climax of elimination, the first to go are those with too few leaves in the light. Among other causes this may be due to shortness, distortion, slow growth, or accentuated crowding. There are more weaklings and distorted trees at this age than at any other, and in their removal comes the critical stage in spacing evolution; for removal of the very old trees above results in intensified elongation and more rapid destruction, since the spacing interval is increased 20–100 times before the third life age is reached. In general, the sapling race is not only a struggle for life by vertical elongation, but it is one in which the time element is crucial.

Having reached the third age, the tree is nearly immune from lateral competition, the permanent stand being formed here. Future struggles are against rot, parasites, wind, and weather, both root and branch systems now being amply competent to maintain life processes. Since the tree's juniors must be limited to what it

cannot use, survival remains with the soundest and best developed. The final picking off in ages 3 to 5 seems slight. In the last age the result of unequal battle with parasites comes out and all fall in turn. It is the rare exception that remains to the last age, one of 100,000 seedlings that have lived and died within its present sphere of influence (GLEASON). In the last age beech is largely replaced by maple in most localities, so that a pure maple-hemlock stand is found in places.

Seasonal periodicity is shown, for example, in the synfolium, present only during summer and part of spring and fall. Each fall it joins the preceding synfolia in the dead leaf layer, thus proving how little actual solid was in it. Chromatic periodicity is more accentuated than in Illinois. The synfolium is yellowish green in spring, quickly turning to the darker green retained through the summer. In fall the birches turn yellow and many maples scarlet. Growth periodicity is shown in the alternating periods of relatively slow growth and active elongation (especially of saplings), according as the inhibition of an older generation persists or is removed.

Evidences of dying or death are unobtrusive but ever present. Nature seems very wasteful in her development of adult trees. The number of saplings pinned down by débris is remarkable. Many are thus actively destroyed instead of passively dying for lack of light. It is needless death and destruction that should in large measure be eliminated by scientific forestry, thus obviating the waste of space and light taken to develop useless plants at the expense of those later useful. Below the sapling synfolium is a death layer which bears, aside from the trunks present, many dead and dying branches.

Branches do damage in proportion to their size, the culmination of destruction coming in the fall of an adult tree. Tree or branch fall is primarily caused by basal rotting. Wind, rain, or lightning is usually required to crack the last resistant marginal alburnum of a branch or unbalance the tree (which has a different type of balance from a branch, so that it can break through proportionally much more wood). The big tree rarely catches on others to remain propped for a while. It usually falls without warning, snatching off branches from its neighbors, and pinning down or lacerating

hundreds of young trees and saplings. There is thus left a natural glade to be closed by regenerative succession.

Competition and parasitism are the main causes of death. Destruction of branches at the synfolium base by lack of light is due partly to slower growth, but primarily to disadvantageous position. In old trees the most serious causes of death are boring insects, fungus rot, loss of foliage and branches, and (possibly) decreased vascular efficiency.

The parasites present are mainly insects and fungi. Neither show prominently in the forest, remaining more or less hidden except for fungus sporophores and many adult insects. Forest floor pileate forms are characteristically present, but individually not very abundant. Coons (20) points out that fungi may also be grouped in formations, certain species being characteristic of each type of habitat. Conditions in the climax forest, especially of the lower levels, favor fungus growth by the relative twilight, more equable temperature, and higher humidity prevailing.

Tunneling bark beetles are present, and, because *Tilia americana* L. and *Fraxinus nigra* Marsh. seem more often attacked, the insects may aid in keeping maple and beech dominant. These beetles, being cambium eaters, would seem more destructive than the duramen eaters, such as *Tremex columba* of maple and beech.

Leaf parasites (23) seem rather few. *Rhytisma acerinum* forms black blotches on maple and oak leaves. A similar fungus causes scarlet patches. Mites causing bag formation on the upper surface of maple leaves, and plant lice occur persistently; woolly aphids (*Schizoneura*) blight the alder, but rarely injure the hardwoods; several sorts of leaf-eating *Microlepidoptera* are found that are worst on the birches, while the tent caterpillars (*Clisiocampa*) confine their attention almost exclusively to rosaceous trees. Thus the maple and beech would seem to enjoy relative immunity from the more serious pests, which may aid in their retaining dominance. The débris includes leaves, twigs, branches, trunks, and stumps, most being found on the ground. Arrest is rare for very light objects (leaves and twigs) and for heavy large ones (trees), but for different reasons. The numbers of the different sorts of débris vary inversely with their size. The leaf layer at the ground surface

furnishing protection and humus, is characteristic of the climax forest. Unlike conifer needles, the leaves fuse during the winter into a single tough layer averaging 2–5 mm. thick, thinnest in late summer and thickest in late fall. Its base continually decomposes, adding to the humus below.

Twigs are always abundant on the forest floor; and since the herbage is open they interfere little with it. Their fall is light and they reach the ground soon, being smooth and slender and not liable to catch. They are easily pushed aside by all plants. Branches often remain on the tree for some time after death, but combined action of basal rotting and weather eventually tears them loose. Yet even then one may not fall, at times hanging by a strand of cortex and alburnum that is often remarkably small, or it may catch on the parent or a nearby tree at one of the crotches or lower branches. Usually one large branch is found on every 3–10 sq. m. Annual vegetation can be hurt for but one season, but perennial aerial parts are injured permanently.

The fallen trunk rots slowly, leaving a soil ridge and a narrow lane for many years. Stumps rot as slowly into a low mound, but hemlocks remain standing as giant stubs 10–20 m. tall with the branches lost. Their wood rots until it cuts like putty, but the bark will hold up for many years, being thick and tough, rich in tannin, and not rotted by fungi or eaten by insects. Maples and beeches rarely leave such stubs, except as the result of fungus entrance some distance up the trunk. Those that are left do not stand long.

Lichens are found sparingly on trunks above the sapling synfolium and on exposed trees. They are also seen on the larger branches and are more common on the maples and hemlocks, because the beech affords poor foothold. A year after a big tree falls, however, its bark is covered by a luxuriant and varied growth of foliose lichens, in consonance with the removal of the substratum from a xerophytic to a richly mesophytic environment.

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Floristics

Gates (7) and Coons (20) define many of the societies found in the region discussed here. It is hoped in a later paper to point out the differences observed from the floristic types recorded and described by these authors (1, 19).

Normal type.—This occupied practically all the uplands of the region before clearing. There are 70–90 per cent sugar maple, 5–30 per cent beech, and the hemlock is a constant tree also, running as high as 25 per cent in some localities. Since many of the forests are not strictly undisturbed and hemlock is taken first (for barking), a low percentage or absence of it may be thus explained in some instances. Other trees occur in varying but small proportions, among the more prominent being *Tilia americana* L., *Fraxinus nigra* Marsh, *Acer spicatum* Lam., *A. pennsylvanicum* L., *Ostrya virginiana* Koch, *Betula alba* L. var. *papyrifera* Spach, *Prunus pennsylvanica* L. f., *P. virginiana* L., *Betula lutea* Michx. f., *Acer rubrum* L., *Ulmus fulva* Michx., *U. americana* L., and *Staphylea trifolia* L.

As type of this forest a quadrat in the primary undisturbed forest back of Bay View was taken (500 sq. m. in 20 squares). There were 17 big trees here, averaging 47 cm. diameter, making the average area occupied 29 sq. m.; 8 of these being maple, 5 beech and 4 hemlock, although the hemlock is more numerous than in much of the nearby woods. Below these trees was a fairly open stand of saplings, those over a meter in height numbering 649; of which 57.3 per cent were sugar maple, 30.1 per cent *Acer spicatum*, 6 per cent beech, the other trees present being *Acer pennsylvanicum*, *A. rubrum*, *Ulmus fulva*, and *Fraxinus nigra*. Their average diameter was found to be 1.41 cm.; the average number per square (25 sq. m.) was 32.5. In a square studied near Walloon Lake the number of saplings was 89 and the average diameter 1.9 cm. The larger size and number in the latter square were probably because it had no adult trees in or very near it, while the Bay View quadrat had, so that its saplings had received only part of the light and nutrients that would otherwise be available.

It will be noticed that *Acer pennsylvanicum* and *A. spicatum* are prominent at age 2 in the first quadrat, and also in some of the climax forest. This is a similar phenomenon, but more accentuated

than the one observed by COOPER (4) in regard to the balsam on Isle Royale. These two maples are ecologically of the sapling type; that is, they reach their highest development in a form ecologically equal to the second life age of the sugar maple. Beyond maturity they have such a high death rate that, although often as abundant as sugar maple at the sapling age, they are rarely represented in the third age. GLEASON'S (11) significant tabulation shows *Acer pennsylvanicum* as the dominant tree after clearing. The contrary occurrence from that of the maple is observed in the case of the hemlock, very few seedlings of which are seen in the climax forest, although a fair number of the adults are constantly present; for, because of scattered occurrence of young trees, it is not probable that the species is dying out.

Shrubs are not common through the climax forest. *Cornus alternifolia* L. f. is often seen in the Bay View woods. The characteristic shrubs of the region include *Sambucus racemosa* L., *Ribes Cynosbati* L. (transitions to *R. gracile* Michx. seem to occur), *R. lacustre* Poir. (along Little Traverse Bay), *Lonicera* (*L. hirsuta* Eaton is occasional along Little Traverse Bay), *Taxus canadensis* Marsh, *Rubus Idaeus* L., *R. allegheniensis* Porter, and *Aralia racemosa* L. The last is really an herb, but it is so tall and large that it is ecologically a shrub and occupies the shrub stratum.

The herbage of the climax forest is varied and fairly abundant. The prevernal flora is sun-loving and close, forming continuous masses of foliage composed of few species and many individuals. In the upland woods the dominant species is *Dicentra canadensis* Walp., but in the woods along Little Traverse Bay *Dentaria diphylla* Michx. appears more prominent. Transition forms to the summer flora occur; for example, *Caulophyllum thalictroides* Michx. is prevernal in leafing and flowering, while in fruit it is strictly aestival. *Allium tricoccum* Ait. also has prevernal leaves which die down before the scape appears in early summer.

The summer herbage is more scattered and richer in species, its richness varying with the age of the youngest tree generation. It is shade tolerant, and characterized by about 50 species. Particularly characteristic among them are *Botrychium virginianum* Sw., *Aspidium spinulosum* Sw., *Trillium grandiflorum* Salisb., *Maianthe-*

mum canadense Desf., *Tiarella cordifolia* L., *Geranium Bicknelli* Britton, *Mitchella repens* L., and *Aralia nudicaulis* L. A typical (1 sq. m.) quadrat at Walloon Lake contained *Geranium* 10, *Viola canadensis* 10, *Allium* 9, *Osmorhiza Claytoni* 2, *Galium triflorum* 1, *Dentaria* 2, grass 2, *Botrychium* 1.

At Bay View the herbs and shrubs show something of a tendency to segregation into patches dominated by different types. Three quadrats of a square meter each taken here were: (1) *Tiarella* 92, *Streptopus roseus* 8, *Dentaria* 4; (2) *Taxus* 24, *Dentaria* 8; (3) *Allium* 116.

VARIANTS.—Both xerarch and hydrarch types can be distinguished. The xerarch occurs on high or hilly ground and is both drier and more open. Either hemlock or beech is prominent. The hydrarch type, shown well behind Bay View, is found in valleys and low ground, either occurring along streams or bearing standing water part of the year. The characteristic trees are linden and yellow birch. The herbage is closed and rich, as many as 40 species being found. *Marchantia*, *Equisetum scirpoides* Michx., orchids such as *Listera convallarioides* Torr., *Impatiens biflora* Walt., *Viola canadensis* L., *Glyceria nervata* Trin., *Polygala paucifolia* Willd., *Habenaria* spp., and *Lycopus* spp. are common; but the most typical character is the large number of ferns. Among the more prominent are *Adiantum pedatum* L., *Asplenium angustifolium* Michx., *A. acrostichoides* Sw., *A. Filix-femina* Bernh., *Phegopteris Dryopteris* Fee, *P. polypodioides* Fee, and *Aspidium spinulosum* Sw.

NATURAL CLEARINGS.—Natural glades and openings occur throughout the primary forest. The fall of a tree is followed in a month or so by a rank herbage growth (11), not the fireweed-composite type often following lumbering, but largely composed of naturally native forest path and clearing species. Among these, by the second or third year, spring up suckers and seedlings of maple and beech, mixed with certain clearing tree species, which shade out much of the herbage growth in four to six years. In the healing of the forest gap the clearing trees may be prominent at first, but they are gradually replaced by the maple and beech in course of time. Among the clearing trees are *Prunus pennsylvanica* L., *P. virginiana* L., *Tilia americana* L., *Ostrya virginiana* Koch,

Ulmus fulva Michx., *Fraxinus nigra* Marsh., and *Betula alba* L. var. *papyrifera* Spach. The herbage is of such species as *Aralia racemosa* L., *A. nudicaulis* L., *Dactylis glomerata* L., *Panicum* spp., *Ranunculus abortivus* L., *Solidago caesia* L., *S. canadensis* L., *Osmorhiza Claytoni* Clarke, and *Geranium Bicknelli* Britton. *Rubus idaeus* L. often plays a large part if the clearing is not too small and the seeds are introduced at a time when room is available.

Secondary scrub and interference

TERMINOLOGY SUGGESTED (3, pp. 145–166)

1. Revegetation
   a) Primary: original or primary vegetation of the area.
   b) Secondary: vegetation coming up after removal of primary society.
      (1) Repetitive: secondary succession following course of primary.
      (2) Nonrepetitive: not following primary.
2. Degree of interference
   a) Partial: few adult trees felled.
   b) Incomplete: all adult trees felled.
   c) Complete: all but herbage removed.
   d) Destructive: all vegetation removed; includes areas where refuse is burned off.
3. Recurrence of interference
   a) Simple: occurs once; area left alone thereafter.
   b) Repeated: interim for partial recovery allowed.
   c) Continuous: repeated at short intervals so that no recovery is allowed.
4. Terrain: left clean, dirty (refuse left), or burned
5. Successional phases
   a) Regressive: reversion to an earlier stage, or "lower" floristic type.
   b) Delayed: same stage but individuals of an earlier age.
   c) Static: approximately same stage and life age.
   d) Progressive: succession hastened.
6. Ecological state
   a) Stage: as used for some point in succession of species.
   b) Age: as used for some point in succession of individuals.

XERARCH TREELESS SOCIETIES

The upland herb and shrub floras appear to show five secondary societies.

FIREWEED SOCIETY.—Most of its species are not native. In clearings, particularly those resulting from destructive interference, with dirty or burned terrain, strongly regressive changes occur,

especially where the soil is stirred up or the humus destroyed. It has been said (3, 9, 10) that this new association is not "lower" than a forest, since it is new, but regression can be conceived as meaning return to a stage where less use is made of the space and light available. Furthermore, forest will finally replace such a society, just as it blots out any naturally formed clearing. The change here is toward a physically and physiographically youthful aspect (rejuvenation of COWLES, 6). The surface soil gives the prevailing tone to the society, being gray or yellow, powdery, and nearly free of available water, radiating intense heat on a warm day.

Into this xerophytic habitat comes a clearing flora showing but a limited number of species, among which *Epilobium angustifolium* L. is dominant. Other species of importance are *Sisymbrium altissimum* L., *Erigeron canadensis* L., *Cirsium arvense* Scop., and *Verbascum Thapsus* L. Besides these occur also *Sisymbrium canescens* Nutt., *Lactuca scariola* L., *L. canadensis* L., *L. spicata* Hitch., *Ambrosia artemisiifolia* L., *Gnaphalium polycephalum* Michx., *Erechtites hieracifolia* Raf., *Sonchus asper* Hill., *S. arvensis* L., *Erigeron annuus* Pers., and *E. ramosus* BSP.

Most of these species have a profusion of wind-borne seeds, and they take possession by having seeds there, by resistance to harsh conditions, by rapid growth, and by seeding profusely over the area when once started, thus getting ahead of competitors. They are finally shaded out by saplings. Certain species of *Lactuca* were observed 3–4 m. tall, and while in "young" dry areas a society as scattered as in a desert may be found, in full development this flora can form a positively impenetrable jungle, particularly on hilly ground with a dirty terrain. Few societies in this region can show such a wide variation of form corresponding with as wide a range of environmental conditions.

THORN SOCIETY.—Its species are natives of the region. This society occurs much in natural clearings, but because artificial clearings and cutovers are so much more numerous in this region at present, the thorn flora is found mostly in such places. It is dominated first by *Rubus idaeus* L., which is commonly succeeded in turn by *R. allegheniensis* Porter. The latter can hold a patch for years against saplings when pickers are numerous enough to

keep the trees down; but if the patch be undisturbed, the sapling growth can replace blackberry in a few years (pin cherry in 3 or 4 years). The thorn species are widely sown by animals that eat the fruits, so their armament serves rather for climbing and individual protection than for keeping out animals. *Erigeron canadensis* L. is the commonest holdover from the fireweed flora. Forerunner saplings are also very usually present. Thus this stage serves as transition where herb and tree meet. It can hold a vicinity far longer than the fireweed society.

Fern society.—*Pteris aquilina* L. occurs with some grass on xerarch areas. Because of its flat-topped habit and proneness to form a pure stand of fronds of equal age and height, this fern forms a synfolium at from 40–80 cm. from the ground, the distance varying with age. Bracken is commonly associated with grass sod, coming in after continuous destructive interference in the drier hilly upland and exposed shore hills. Aspen often is found with it, both entering particularly after fire (12). *Myrica asplenifolia* societies of farther south (Little Manistee to Brethren) appear to be equivalent.

Milkweed society.—The species is not native of the region. *Asclepias syriaca* L., while common as a weed, and found in all sorts of societies, also forms a persistent, ubiquitous, and actively invading society of its own on drier upland and lowland areas. Like *Pteris*, it is often associated with grass turf, possibly because of the natural openness of the two societies. It probably enters after more severe and continuous interference than the bracken can endure. Because of its underground rhizome, xerophytic structure, tremendous reproduction, and efficient seed dispersal, it can maintain itself after continued cutting and even plowing. It probably would precede sumac in reclamation of unused upland pastures, and is prominent where interference (and turf?) prevents later successional stages in pasture and grassy upland.

Sumac society.—These species are probably native here. *Rhus typhina* L. and *R. glabra* L. occur in upland pastures, along roads, and in clearings, being primarily a bordering association (thus later than milkweed), occurring more often on closed (and especially clay) soil. It forms a stand 1–2 m. high, much opener than the milkweed, and being taller permits more herbage. Along roads it is often

followed by maple-beech, although the regenerating climax forest can enter at any stage of the upland secondary series. One of the evidences suggesting equivalency of the thorn, fern, and milkweed societies is that sumac can follow any one, and that any of the three can succeed the fireweed flora.

Xerarch tree society

The aspen–white birch–pin cherry society varies much in general form and specific content, so three types (consocies) are found. The dominant trees are *Populus tremuloides* Michx., *P. grandidentata* Michx., *Betula alba papyrifera*, and *Prunus pennsylvanica* L.

Pin cherry–birch.—The birch may be absent. Pure pin cherry stands in particular occur in upland and middle level clearings following the thorn society. They can spring up suddenly. In spite of good light the lower branches remain slender and die early. The mode of growth is the arboreal expression of the clearing society type; all are also soft wooded. The pin cherry is ecologically peculiar in being strongly excurrent, with elongate form and filiform type of branches, wasting the minimum of tissue on laterals and trunk diameter. This gives it great power of vertical elongation, an aid in competition for place in clearings, but makes it short-lived; so in time it must give way to longer-lived hardwoods. Thus the forest of this type is fairly open, with good herbage.

Aspen–pin cherry.—This is a dry open xerarch type found along shore, especially on the ridge back of the Nipissing cliff. Often half the trees will be dead and the remainder equally divided between the two species. Herbage is scant or none, and dead twigs and branches are thick below. Such a stand is far opener than either maple-beech or cedar forest, although similar to the cedar in number of dead trees.

Aspen-Pteris.—This is found more on dry levels inland. The small-toothed aspen dominates (90 per cent or more); the large-toothed aspen is prominent; and some birch may be found. Being secondary, the herbage below the bracken synfolium suggests a high type of primary forest. The following species are found: *Gaultheria procumbens* L., *Cornus canadensis* L., *Lonicera hirsuta* Eat., *Corallorhiza* spp., and *Lycopodium tristachyum* Pursh. The

bracken seems to interfere little with this herbage, in fact may protect it. They occupy a different level and seem complementary (22).

The preceding types are alternative. Which enters depends on soil conditions, topography, and seeds present. Aspen succeeds best after fire and in higher dry ground. Pin cherry seeds are bird-scattered (and fertile longer), thus being more apt to reach a favorable place. The xerarch tree society is able to enter in many cases where climax forest is sufficiently cleared, either with or without intervention of treeless stages. The greater the degree and the quicker the recurrence of interference, the more likely regressive changes are to occur, its amount and the environment determining whether the ensuing secondary state be repetitive or non-repetitive, and, if the latter, whether it be thrown back to tree, shrub, or herb stage (the farthest being the fireweed semi-desert), that is, the degree of rejuvenation.

Regeneration of the climax forest may be speedy, hardwood saplings following a mixture of fireweed and thorn. This results in a remarkable floristic mixture. Behind Bay View a nearly pure stand of red maple has been formed after cut over. At Walloon Lake the beech is apt to dominate in the regenerating climax forest. In other places *Acer spicatum* and *A. pennsylvanicum* are important. If the xerarch tree society takes charge of a district it may be followed by yellow birch and elm (18) before the climax supervenes. Grass turf may prevent tree entrance (11, 12), but it would appear that milkweed and sumac, at least for dry upland, could replace it.

Discussion

The upland societies here studied show that most of the area of this character was occupied before settlement by climax forest. The forest itself (as any climax) is static in species but dynamic as to individuals, so that the climax is not final but recurrent. Five life ages may be singled out in this forest, each with its own dimensions and ecological characters. Thus the sapling age shows maximum increase in size for given decrease in number per unit area, so that competition between trees of equal age is keenest here.

For the foliage layer is coined the name synfolium, and its development and ecological significance are analyzed. In connection with mortality, it is pointed out that very many saplings are pinned down by débris, and thus actively destroyed instead of passively dying.

The study of Kent County, Michigan, by LIVINGSTON (18) shows five societies, while the writer has distinguished four here. While this might be interpreted as meaning Kent County was not so far advanced, it must be remembered that: (1) oak and hickory play more important rôles in succession in the Grand Rapids area than at the north end of the southern peninsula; (2) LIVINGSTON recognizes three societies containing oak, and two with maple; in this region the four primary types tend to be mutually exclusive; (3) LIVINGSTON uses herbs as well as trees in definition of his societies, which the author has not felt justified in doing for this region as yet.

In examining the distribution of the secondary societies, it seems probable that the hypothesis laid down by LIVINGSTON holds in large measure, but it may be that this is only for societies enduring rather small differences in moisture retaining power of the soil; for the fireweed society (secondary) is able to endure a wide range in this particular, being shaded out, but not dried or drowned out.

Throughout this region the response of the plant societies to interference and changed environment has been adaptive, in so far as their constitution allowed. Some natural societies, such as the blackberry, are fitted to survive in partly wild areas. Others can invade the fields in competition with the crops. Characters required are quick entry, speed of vertical growth, quickness of fruiting after germination, quantity of seed production, and efficiency of distribution; for a given society may be here today and gone tomorrow, plowed under.

The best example of the weed type among the societies previously discussed is the fireweed society, which contains species that are being rigidly selected by man in his fight against them. These plants are likely to survive long after the maple-beech society is banished to the wood lot and city parkway; for evolution is toward

the herbaceous annual type (as pointed out by SINNOTT and BAILEY in past evolution also), as best suited to the mobile environment furnished.

The author desires to extend his thanks to Professor HENRY C. COWLES, of the University of Chicago, whose help and encouragement have made the completion of this work possible. He also wishes to thank Mr. CHARLES W. FALLASS, of Petoskey, and Professor CHARLES H. SWIFT, of the University of Chicago, for their cooperation during the progress of the work.

OAK PARK, ILL.

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FIELD AND LABORATORY STUDIES OF VERBENA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 257

(WITH PLATES VI–IX AND TWENTY-SIX FIGURES)

M. KANDA

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FIELD AND LABORATORY STUDIES OF VERBENA

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M. KANDA

(WITH PLATES VI–IX AND TWENTY-SIX FIGURES)

Introduction

In GRAY'S *New Manual of Botany* (edition of 1908), 8 species of *Verbena* are described as occurring in the Eastern United States. These are classified into two sections, of which the first is further subdivided into three groups. Five of the 8 species grow wild in the vicinity of Chicago, namely, *Verbena urticaefolia* L. and *V. bracteosa* Michx., belonging to the first and third groups respectively, and *V. angustifolia* Michx., *V. hastata* L., and *V. stricta* Vent. to the second group. These three last named species occur abundantly at Stony Island, a southern suburb of Chicago, where the conditions of prairie, damp, and dry ground are met with successively as one proceeds from the north to the south end of the locality. Here the three forms grow in their characteristic ecological situations: *V. stricta* on the prairie, *V. hastata* in damp low places, and *V. angustifolia* on high dry ground. On examining the *Verbena* plants, one is rather surprised to find that there are many intermediate forms which can scarcely be assigned to any of the three species with certainty. The question arises, therefore, as to whether they are hybrids or mutants of the three species.

The present work was undertaken to determine whether or not there are any cytological differences in the fertilization phenomena and early stages of development between these forms. The results were rather negative as regards the genetic nature of the intermediate forms; that is, with slight exceptions, no significant differences were found between them. Many of the observations upon the embryonic development, however, are sufficiently interesting to merit description. These will therefore constitute the chief subject matter of the present paper, such facts and suggestions as I am able to present regarding the origin and nature of the intermediate forms being added at the close.

This work was carried on at the Hull Botanical Laboratory, University of Chicago, under Professor CHARLES J. CHAMBERLAIN, to whom I wish to express my sincere thanks for suggesting the problem, and my appreciation of his kind advice throughout the progress of the work. My acknowledgments are also due to Professor JOHN M. COULTER for his kindness in placing the conveniences of the laboratory at my disposal.

Taxonomic observations

Although one can easily recognize the specific characters of the original species, *V. angustifolia*, *V. stricta*, and *V. hastata*, it is impossible to arrange the forms intermediate between them in a linear series with regard to all of their contrasting characters. In other words, all of the characters do not vary in the same direction, so that if one distributes them among the original species with reference to one character, a different distribution would be required for some other character. Examples of the 3 species and the 6 intermediate forms which I was able to collect are given in figs. 1–9 (pl. VI). Figs. 1, 3, and 7 are *V. angustifolia*, *V. stricta*, and *V. hastata* respectively, and the others are the intermediates arranged between the 3 species in accordance with their degree of similarity to them, as nearly as this could be determined. I have attempted to represent diagrammatically the morphological relationship between the originals and the intermediates by the triangle shown in text fig. 10; the numbers on the triangle refer to the figures in plate VI. The three apices (1, 3, 7) indicate the three original species, and the points along the sides of the triangle show the probable position of the intermediate forms with reference to them. For example, 4 is believed to be nearer to 3 than to 7, and 5 is probably about midway between 3 and 7. The contrasting characters of all of the forms are given in detail in table I.

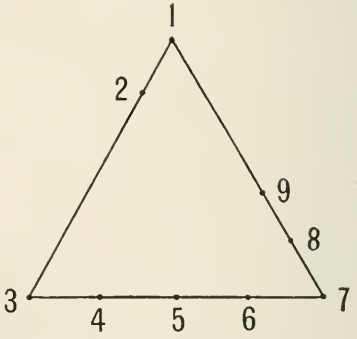


FIG. 10.—Diagrammatic representation of morphological relationship between originals and intermediates.

TABLE I

No.	Height in cm. in average	Stems			Leaves					Spikes		Corollas		Bracts
		Form of cross section	Branched or not	Stout (+) or slender (−)	Breadth in cm. in average	Length in cm. in average	Petiolated or sessile	Serrature double (+) or single (−)	Hair thick (+) or not (−)	Thick (+) or filiform (−)	Clustered (+) or loose (−)	Color	Size	Long (+) or short (−)
1...	25.2	Quadrangular	Apical part only	−	0.7	5.1	Sessile	−	− and rough	+ slightly	−	Pale purple	Medium	− and leafy
2...	30.0	Quadrangular	Apical part only	− but stronger than former	2.7	7.0	Sessile	−	+ and rough	+ than preceding	−	More violet than preceding	Slightly larger	+ and leafy
3...	88.0	Round	Not except sometimes at apical part	+	4.5	8.5	Sessile	+ heavy	+ and woolly	+ strongly	+	Purple	Large	+ and slightly leafy
4...	120.0	Round quadrangular	Not except sometimes at apical part	+	5.0	12.0	Short petioles	+ heavy	+ and slightly woolly	+	+	Purple	Large	−
5...	99.9	Round quadrangular	Not	+	3.2	9.0	Long petioles	+ slight	+ and rough	+	+ slightly	Lilac	Large	+ and narrow
6...	120.0	Quadrangular	Rare	+	2.7	8.5	Short petioles	+	+ rough and stiff	+ slightly	+ slightly	Purple	Slightly larger	−
7...	187.0	Quadrangular	Apical part only	+	2.5	11.4	Long petioles	+ slight	+ short	−	+	Purple lilac	Small	+ and narrow
8...	120.0	Quadrangular	Apical part only	Somewhat −	1.7	10.0	Long petioles	−	+ short	−	+ slightly	Pinkish purple	Slightly larger	−
9...	82.5	Quadrangular	Apical part only	Somewhat −	3.0	9.5	Long petioles	−	+ short	−	+	Pinkish purple	Slightly larger	−
1...	25.2	Quadrangular	Apical part only	−	0.7	5.1	Sessile	−	− and rough	+ slightly	−	Pale purple	Medium	− and leafy

It is necessary to consider whether or not the differences between these plants might not have been induced through adaptation and response to the local conditions in which each type may happen to be growing. Such an influence of local factors can be recognized at Stony Island in different degrees; thus, for instance, while the color of the flowers of *V. hastata* varies greatly with individuals, without reference to the conditions of the habitat, the shape and texture of the leaves of this species are plainly responsive to the surroundings, those plants growing in dry places having narrower and stiffer leaves than those inhabiting wet situations.

I believe I have eliminated this possibility in selecting my materials, and those which I regard as intermediate forms are not cases of modifications due to individual differences or adaptation to local conditions. Thus I have found forms 1 and 2 growing under the same external conditions at one location; forms 4, 5, and 6 growing together at another place; and forms 8 and 9 growing at a third spot.

Cytological observations

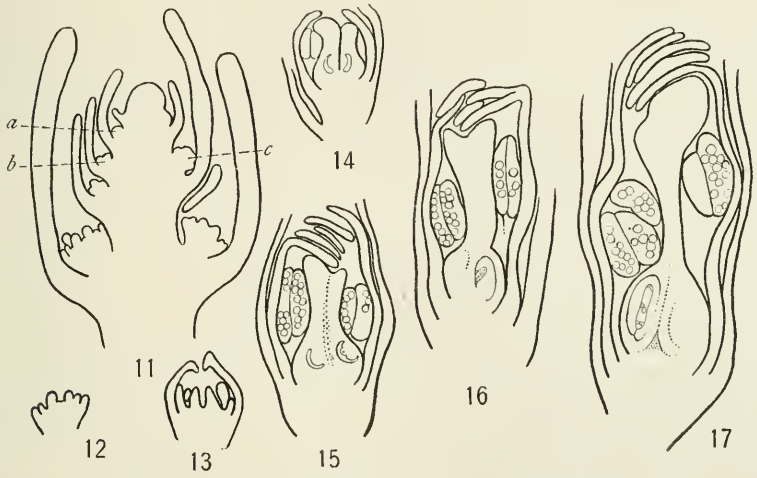
Material and methods

The spikes of *V. angustifolia* (fig. 1), *V. stricta* (fig. 3), and *V. hastata* (fig. 7), and the form intermediate between *stricta* and *hastata* (fig. 5) were collected during July and August 1918 at Stony Island. The apical part of the spikes, the pistils, and the young fruits in different stages of development were fixed in chrom-acetic acid and corrosive sublimate–acetic acid solutions, the former giving the best results. In the case of the pistils and fruits, it was found advantageous to pick off carefully or partially remove the calyx tubes, as they interfered with the rapid penetration of the fixing fluid. Sections of the apical part of the spikes were cut 5, 10, and 15 μ in thickness; pistils and young plants, 5 and 7.5 μ. Flemming's triple stain and iron alum haematoxylin were used, the former giving quite satisfactory results.

All of the four forms mentioned were examined in more or less complete series. *V. angustifolia* is chosen as a type for the purposes of description, but most of the statements are applicable to the others also, and they will be mentioned specifically only where differences between them make a separate discussion necessary.

Development of flower

The first evidence of the formation of flowers is the appearance of papillae in the axils of the bracts (fig. 11*a*); these papillae are the primordia of the receptacles of the flowers. The outline of the receptacle soon becomes angular through the upward growth of four hemispherical protuberances from its distal surface (fig. 11*b*), and soon afterward its base produces a ring-shaped outgrowth (fig. 11*c*). The former develop into the stamens, and the ring immediately afterward separates into the corolla and the calyx



Figs. 11–17.—Floral development in *V. angustifolia;* ×35

tube (fig. 12). The appearance of the carpels is indicated by a broadening of the receptacle (figs. 12, 13).

In fig. 13 the calyx tube has begun to curve inward over the top of the flower. Within this the corolla tube, the hemispherical young stamens and the two carpels appear in succession. Their later stages are shown in figs. 14–17.

Development of megaspore and embryo sac

When the ovule has reached the stage shown in fig. 15, the subepidermal megaspore mother cell that terminates the axial row of the nucellus can readily be distinguished from the surrounding cells through its larger size and large nucleus (fig. 18). The

megaspore mother cell and its nucleus with a prominent nucleolus continue to increase in size (fig. 19). Two divisions then occur which result in the typical formation of a row of four megaspores (figs. 20, 21); this takes place when the ovule is about at the stage represented in fig. 16. The innermost of the four megaspores is the largest, and is destined to develop into the embryo sac (fig. 22).

Successive stages in the development of this basal megaspore, accompanied by the destruction of the other three megaspores, are shown in figs. 22–25. The nucellus, consisting of a single layer of cells, surrounds the row of megaspores (fig. 21). It eventually becomes so distended by the enormous expansion of the developing embryo sac that it ruptures, and the ruptured nucellus is then carried downward as a cap on the growing embryo sac, as was previously described by MOTTIER (14) in *Arisaema*, CALDWELL (1) in *Lemna*, and MERRELL (13) in *Silphium*. In the next stage (fig. 26) the embryo sac lies free in the space between the funiculus and the integument, and the yellowish-brown remnants of the nucellus are observable capping the micropylar end of the sac.

27

FIG. 27.—*V. angustifolia:* mature pistil with mature embryo sac; ×35.

The phenomena of the enlargement of the sac, the division of its nuclei, and the destruction of the cells of the nucellus do not occur simultaneously, but these processes take place at different rates. The development of the megaspore and the fate of the nucellus are exactly the same as described by MERRELL for *Silphium*.

When the embryo sac reaches maturity (fig. 26), taken from an ovary in the stage represented in fig. 27, the sac is several times larger than it was when inclosed in the nucellus, very slender in shape, and always constricted just above the egg apparatus. The egg apparatus seems to be typical. The nucleus of the egg is several times larger than the nuclei of the synergids and contains

in the resting condition a fine chromatin network and a large, often vesicular, nucleolus. After the fusion of the polar nuclei, which occurs near the middle of the sac (fig. 25), the resulting endosperm nucleus approaches the egg apparatus. At this time, as shown in fig. 26, the endosperm nucleus still possesses two nucleoli, evidences of its binucleate origin, and is considerably larger than the egg nucleus. It is frequently in contact with the egg. There are three very small but typical antipodal cells.

The nutritive jacket surrounding the embryo sac of *Verbena* usually consists of a single layer of cells derived from the inner epidermal layer of the integument, and it develops especially at the micropylar end, investing the egg apparatus of the embryo sac. The cells of the jacket have conspicuous brownish contents, among which are numerous starch grains. Rather frequently a portion or portions of the jacket cells inclosing one or more grains of starch protrude into the embryo sac.

DEVELOPMENT OF MICROSPORES

At the stage shown in fig. 14 the hypodermal archesporial row is distinguishable, and the succeeding stages follow the usual course of development (figs. 28, 29). There may be only a single longitudinal row of spore mother cells, but one or two longitudinal divisions of the primary sporogenous row may take place (fig. 30).

The pollen mother cells within a loculus do not divide quite simultaneously, so that several different stages of the reduction division may be found among them (figs. 31–33). It is rather difficult to count the number of chromosomes in this species (*V. angustifolia*) because they are remarkably small and slender, but it was ascertained that 8 is the $2x$ number. In the second maturation division the two spindles usually lie across each other as in fig. 33.

In *V. angustifolia* there are two different types of tetrad formation. In the one case the peripheral cytoplasm of the pollen mother cell is left over to form a wall for the tetrad, this wall subsequently disintegrating (figs. 34, 35), while in the other case the entire mother cell is utilized in the formation of the tetrad (fig. 36). Figs. 37–41 give successive stages in the development of

the pollen grains. The wall of each microspore gradually thickens and sometimes a great many starch grains may be observed in the interior (fig. 39). Cases of accumulation of starch grains in the pollen have been reported by MURBECK (15), ISHIKAWA (11), and others. In *Oenothera* ISHIKAWA states that "the plasm containing starch grains in the pollen tube is poured into the attacked synergid," but in this case no starch is present in the pollen tube (fig. 42). A large vacuole appears in the pollen grain for a time (fig. 40), but it soon fades away and the first vegetative cell is cut off (fig. 41). More advanced stages could not be observed, as the contents and wall of the pollen grains become extremely dark in color. While these changes are occurring, the tapetum and middle layer disintegrate.

FERTILIZATION

It is very difficult to obtain clear pictures of the stages in which the male nuclei are on the point of fusing with the egg cell and the endosperm nucleus. In the first place the egg apparatus is rendered very indistinct through the presence of deeply staining cytoplasmic substances around it. I believe this deeply staining material is the result of a concentration of the cytoplasm and the inclusion within it of nutritive substances destined for the endosperm. The abundance especially of starch grains around the egg apparatus greatly confuses its appearance with the gentian violet stain. Secondly, the synergids seem to be more ephemeral in *Verbena* than in other plants, and soon become converted into a tenacious mucus-like material. This material from the disorganized synergids also stains very deeply. Thirdly, when the pollen tube enters the egg apparatus, a part of the disorganized nucellar cap penetrates into it with the tube and always gives rise to a figure of peculiar shape and staining properties (figs. 42–44, 46). MERRELL states that in *Silphium* "the pollen tube passes along the outside of the cap which usually crowns the embryo sac and enters the sac just beyond its free margin." In *Verbena*, however, the pollen tube, entering the sac at the micropylar end, thrusts itself through the nucellar cap (fig. 42), just as in *Lemna*, described by CALDWELL.

Figs. 43 and 44 show stages of fusion of the male and female nuclei. In fig. 43 one of the male nuclei is in contact with the egg and the other with the embryo sac nucleus, and in fig. 44 one of the male nuclei has fused with the egg nucleus.

In connection with the fertilization process it should be reported that at this time a proteid-like substance makes its appearance in the cavity between the carpels and ovules (figs. 26, 27). This material forms a network, probably as the result of coagulation by the fixing agent, and stains deeply with cytoplasmic dyes. The only suggestion which can be offered as to the function of this substance is that it may be related to the nutrition of the pollen tube, since it appears just before fertilization and disappears shortly after that process is completed.

Formation of endosperm

After fertilization the primary endosperm nucleus moves toward the center of the embryo sac, and its first division takes place there. This division is followed by the formation of a wall which divides the sac into two approximately equal chambers, the micropylar and the antipodal chambers (figs. 45, 46). Such a formation of a two-chambered embryo sac has been observed in many plants, both monocotyledons and dicotyledons, by Hofmeister (10), Schaffner (17), Campbell (2), Guignard (6), Hall (8), Murbeck (15), Cook (3), and others. Several other cases are mentioned by Coulter and Chamberlain (4).

The nucleus of the micropylar chamber gradually changes its position, moving toward the middle of the chamber, and soon afterward produces a great many free nuclei (figs. 46, 47), around which walls are subsequently formed, beginning at the micropylar end. This mode of development of the endosperm corresponds to the third type in Hegelmaier's (9) classification. Twelve chromosomes, that is, the $3x$ number, were often counted in these nuclear divisions. The nucleus of the antipodal chamber also moves toward the center of that chamber, and increases in size, but does not undergo division for a long time (figs. 46, 47). The antipodal chamber elongates like a haustorial tube, extending to the chalazal extremity of the ovule, sometimes becoming exceedingly curved.

Figs. 48 and 49 illustrate two parts of the same embryo sac; the endosperm tissue is seen to be fully formed in the micropylar chamber, while the antipodal chamber is still uninucleate.

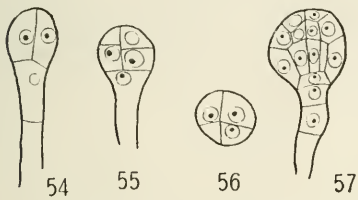
A large amount of starch is present in the embryo sac, as was also observed by GUIGNARD (7) (*Cestrum*), D'HUBERT (5) (Cactaceae), WEBB (18) (*Astilbe*), and LLOYD (12) (*Calicium*). This is observable not only a little before fertilization, but more especially after fertilization has occurred (figs. 43, 44, 46). Fig. 46 shows starch not only in the micropylar and antipodal chambers, but also even in the egg cell. It is evident that the starch grains in the micropylar chamber are always larger than those in the antipodal chamber. These starch grains are naturally closely related to those in the nutritive jacket. I have already mentioned that jacket cells loaded with starch grains may protrude into the sac. Sometimes one gains the impression that the starch grains have entered the sac through the destruction of the thin walls of the jacket cells. Such a direct transfer of starch, however, is hardly to be credited, partly because there are many fewer grains in the sac than in the jacket, but mainly because the walls of the jacket cells seem to be composed of very resistant material, since they persist for a long time apparently intact. In the *V. hastata* material I found occasionally an entire absence of starch grains in the jacket cells, and in such cases the development of the embryo sac is always remarkably retarded, and the egg apparatus is absent (fig. 50).

The further development of the endosperm is the same as in *Sagittaria*, described by SCHAFFNER (17). While the micropylar chamber is becoming filled with walled endosperm tissue through free nuclear division, the enlarged nucleus of the antipodal chamber still remains undivided. Sometimes it divides once or twice (fig. 51), forming two or three free nuclei which enlarge enormously. Meantime the endosperm tissue continues to develop, finally extending from the micropylar chamber into the antipodal chamber, forcing the large cell which occupies the antipodal chamber up to the antipodal end. At about this time the antipodal cells disintegrate (fig. 52). The large cell at the antipodal end of the chamber gradually diminishes in size, and finally disappears.

In COULTER and CHAMBERLAIN'S book (4) it is stated that "the endosperm is said to develop only in the antipodal chamber in *Loranthus*, Vacciniaceae, Verbenaceae, etc." This statement should be corrected as far as it concerns the various species of *Verbena* which I have studied.

DEVELOPMENT OF EMBRYO

The proembryo divides in two by a transverse wall and remains without further change for a long time (fig. 49). It then elongates, with accompanying divisions, reaching a condition like that illustrated in fig. 53, where it is a filament of varying length, consisting of several cells. The apical cell of the filament then divides longitudinally (fig. 54), followed by another longitudinal and a transverse division in either order, resulting in an octant stage (figs. 55, 56).



FIGS. 54–57.—*V. hastata:* successive stages of development of embryo; fig. 56, apical view of stage in fig. 55; ×400.

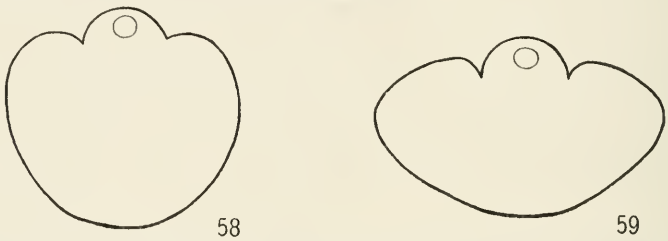
The dermatogen, periblem, and plerome layers are next differentiated in the embryo (fig. 57), which now occupies the end of a long suspensor. The appearance is identical with that of *Capsella*.

Relationship of intermediate forms

COOK, comparing two species of *Sagittaria*, *S. variabilis* and *S. lancifolia*, says: "With such striking external differences one would naturally expect equally interesting internal differences, but to my surprise I found the development of the embryo sac and embryo of *S. lancifolia* practically the same as had been described by SCHAFFNER for *S. variabilis*." I was equally surprised on comparing the forms of *Verbena*. I selected as the intermediate form for comparison with the original species the type designated in the earlier part of this paper as no. 5 (see fig. 5), because it is one of the most abundant of the intermediates and because it seemed to be halfway between *V. stricta* and *V. hastata*. In the following account the morphological and cytological characters of this intermediate are compared with those of the three species.

The flowering period of *V. angustifolia* comes earlier than that of *V. stricta*, *V. hastata*, and the intermediate form between them, so that the last three flower at the same time. For this reason one would expect that intermediate forms between *V. angustifolia* and the other two species would be rather rare, while those between *V. stricta* and *V. hastata* would be more common, if these intermediate forms are really hybrids. As a matter of fact, the relative abundance of the intermediates corresponded to the expectation.

The young ovule of *V. hastata* at the stage in which the megaspore mother cell first makes its appearance (fig. 15) is rounded (fig. 58), while that of the other three forms is somewhat flattened, as indicated in fig. 59. The young ovule of the intermediate form is therefore similar to that of *V. stricta*.



FIGS. 58, 59.—Diagrammatic outline of young ovule: fig. 58, *V. hastata;* fig. 59, other 3 forms.

The size of the mature embryo sac varies considerably within each species owing to individual variations, but an approximate comparison of its size at the same stage in the four forms can be made without difficulty. The following table gives the average length of 12 embryo sacs of the four forms at three different stages.

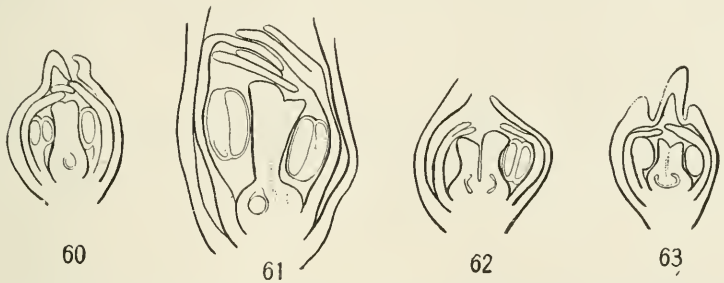
TABLE II

Name	*V. angustifolia*	*V. stricta*	Intermediate form between *V. stricta* and *V. hastata*	*V. hastata*
Fig. 26 stage.....	0.260 mm.	0.225 mm.	0.185 mm.	0.185 mm.
Fig. 51 stage.....	0.500	0.460	0.390	0.310
Fig. 56 or 57 stage	0.460	0.540	0.360	0.340

The breadth of the sac in all cases is about 0.02–0.03 mm. The figures show that with regard to the length of the embryo sac the intermediate form resembles *V. hastata* more than it does *V. stricta*.

At the time of the first mitosis of the microspore mother cell the flower buds of the 4 forms are in different stages of development. As shown in figs. 60–63, the buds of *V. angustifolia* and *V. hastata* are in a relatively young stage when this event occurs, those of *V. stricta* in a much later stage, and the intermediate form at a stage between these two. In respect to this character, then, the latter occupies an intermediate position.

As described in a preceding section, tetrad formation occurs in *V. angustifolia* in two different ways, with or without persistence of a rim of cytoplasm from the mother cell. In *V. stricta* the cytoplasm always persists in this manner, forming, even at the first mitosis of the microspore mother cell, a deeply stained border



FIGS. 60–63.—Comparison of florets at time of first mitosis in pollen mother cells: fig. 60, *V. angustifolia;* fig. 61, *V. stricta;* fig. 62, intermediate form between *V. stricta* and *V. hastata;* fig. 63, *V. hastata;* ×35.

around the central portion where the mitosis is occurring (figs. 64, 65). In *V. hastata* no such cytoplasmic border is ever formed around the microspores, but all of the cytoplasm of the mother cell is utilized in the production of the pollen grains. The intermediate form is like *V. hastata* in this regard (figs. 66–68).

V. angustifolia has 8 chromosomes as the $2x$ number. A late prophase and metaphase of the first reduction division in this species are shown in profile view in figs. 69 and 70. The other 3 forms have 12 chromosomes as the $2x$ number. A metaphase of *V. stricta* and an early anaphase of the intermediate form from the side and end are illustrated in figs. 71–74. I regret that in *V. hastata* I was unable to find just the same stage to compare with these, as all of my material of this species is either a little too early or too

late. It is safe to conclude, however, that 12 is also the $2x$ number for this species, since in the early telophase of the first division (fig. 75) 6 chromosomes are clearly present at each pole of the spindle. I have further often counted 12 chromosomes in all of the forms except *V. angustifolia* in the anaphase stage in young locular cells of anthers, and 18 chromosomes, the $3x$ number, in the endosperm cells. The behavior of the chromosomes of the intermediate form in mitosis is entirely normal, and like that of the original species. No such abnormalities as were described by ROSENBERG (16) in *Drosera* hybrids can be recognized.

Owing therefore to the unfortunate fact, which could not be foreseen, that both of the original species selected for comparison with a form intermediate between them have the same number of

FIGS. 69–75.—Mitosis of pollen mother cell: figs. 69, 70, *V. angustifolia;* figs. 71, 72, *V. stricta;* figs. 73, 74, intermediate form between *V. stricta* and *V. hastata;* fig. 75, *V. hastata;* ×1500.

chromosomes, cytological observations upon them do not serve to settle the question as to whether the intermediate form is a hybrid or not. It is clear that the intermediate form does not differ cytologically from the original forms, and that its mitotic behavior is entirely normal. These facts, if they have any significance at all, tend to suggest that the intermediate is not a hybrid, but rather a mutant of one or the other of the original species. This could be determined only by breeding it through several generations and observing whether its characters are fixed or not.

Cytological studies of the forms intermediate between *V. angustifolia* and the other two species might have yielded more definite results, because it differs from them in the number of its chromosomes. Unfortunately I did not collect any material from these forms, as they are relatively rare.

Summary

Several intermediate forms were found between three species of *Verbena* which grow on Stony Island, *V. angustifolia* Michx., *V. stricta* Vent., and *V. hastata* L., which can be arranged taxonomically between the three species in question. Embryological and cytological studies were made on the three species and on one of the forms intermediate between *V. hastata* and *V. stricta* in order to determine the genetic nature of the intermediate.

From the cytological point of view, nucellar cap, nutritive jacket, and chambered embryo sac are pointed out as the characteristic features of these forms. The reduced number of chromosomes is 4 in *V. angustifolia* and 6 in the other three.

It was not possible to decide from the cytological studies whether the intermediate form is a hybrid or not, since both of the original species from which it might be supposed to have sprung were found to have the same number of chromosomes. The chromosome behavior of the intermediate was like that of the two species and entirely normal. Some of its developmental characters are intermediate and some are similar to either *V. stricta* or *V. hastata*.

NORMAL COLLEGE
HIROSHIMA, JAPAN

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EXPLANATION OF PLATES VI–IX

Figs. 10–17, 27, 54–63, 69–75 are in the text; all the others in the plates. All drawings were made with an Abbé camera lucida at table level. Figs. 11–17, 27, and 60–63 were drawn with Zeiss compensating ocular no. 4 and Spencer 16 mm. objective; figs. 18–25, 28–41, and 64–68 with Reichert ocular no. 18 and Spencer 4 mm. objective; figs. 26 and 42–53 with Zeiss compensating ocular no. 4 and Bausch and Lomb 1/12 oil immersion objective; figs. 69–75 with Reichert ocular no. 18 and Bausch and Lomb 1/12 oil immersion objective. Text figures reduced one-half, plates nearly two-thirds in reproduction. The original magnification will be specified for each figure in the plates.

PLATE VI

All figures reduced five-twelfths.

Fig. 1.—*Verbena angustifolia* Michx.

Fig. 2.—Taxonomically intermediate form between *V. angustifolia* Michx. and *V. stricta* Vent.

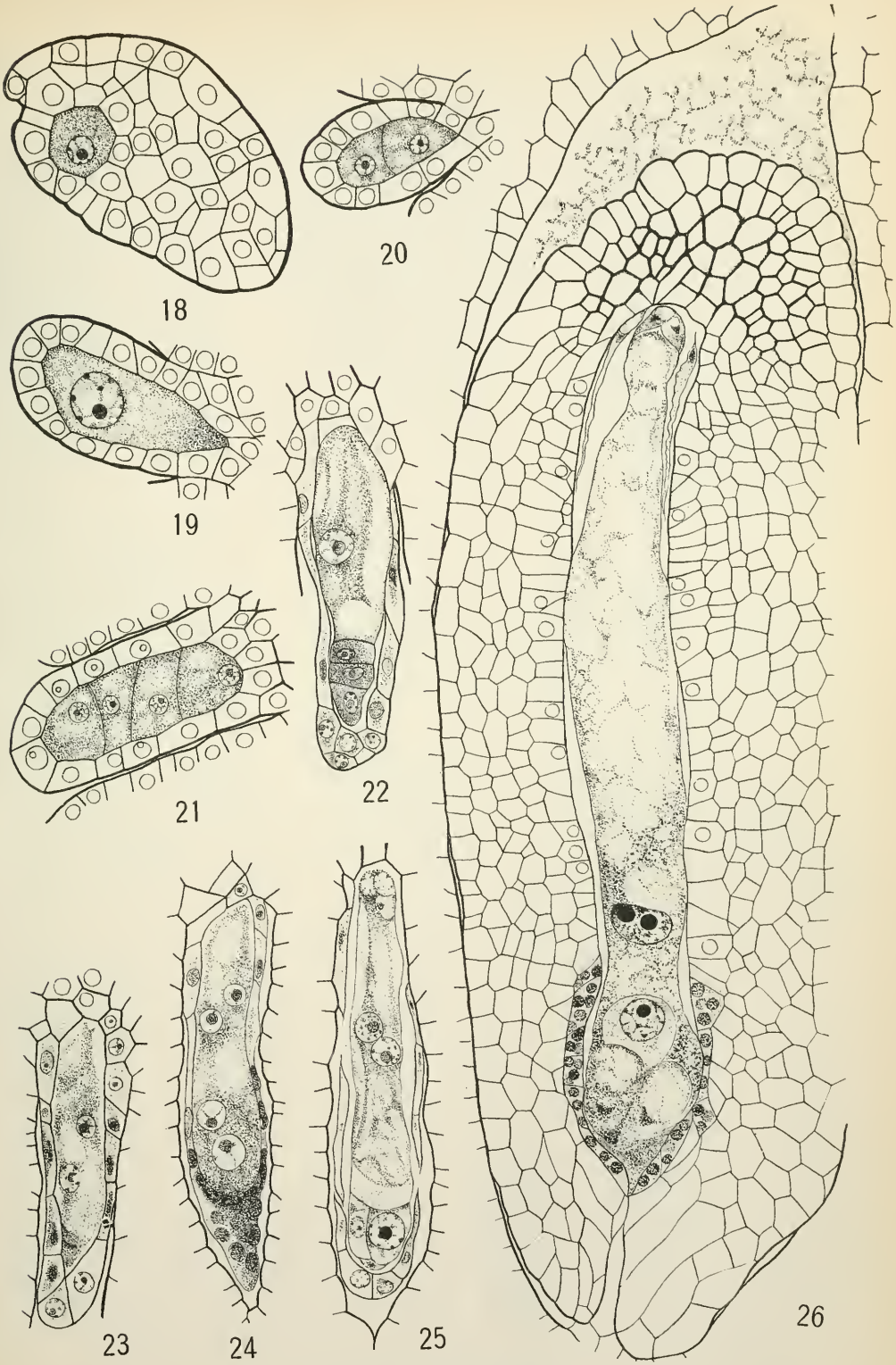
Fig. 3.—*V. stricta* Vent.

Figs. 4–6.—Taxonomically intermediate forms between *V. stricta* Vent. and *V. hastata* L.

Fig. 7.—*V. hastata* L.

Figs. 8, 9.—Taxonomically intermediate forms between *V. hastata* L. and *V. angustifolia* Michx.

KANDA on VERBENA



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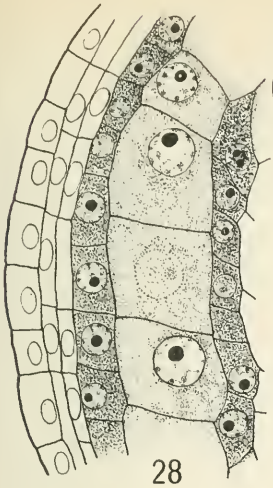
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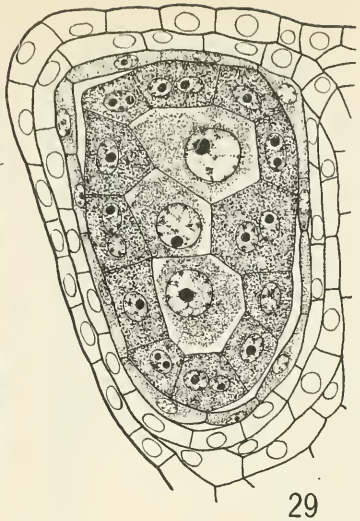
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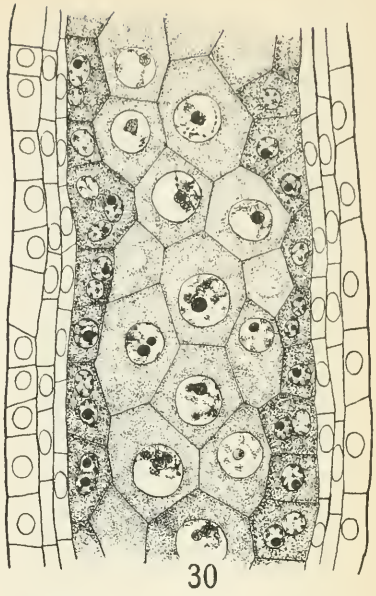
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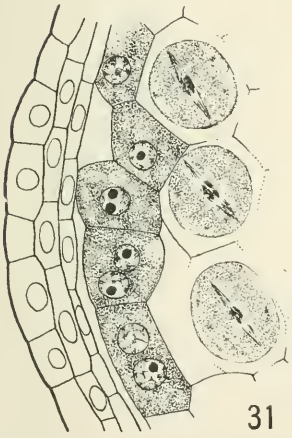
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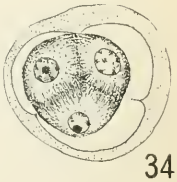
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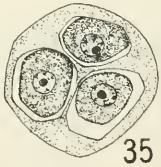
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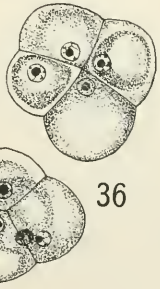
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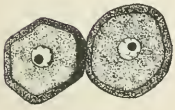
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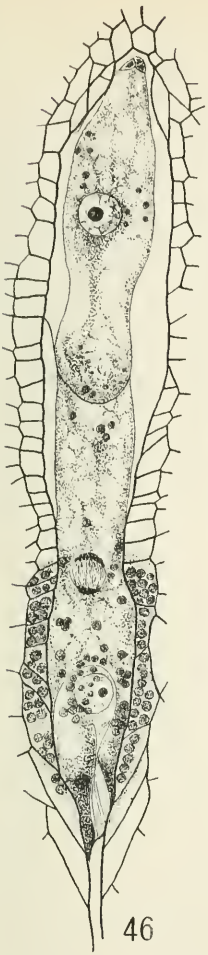
KANDA on VERBENA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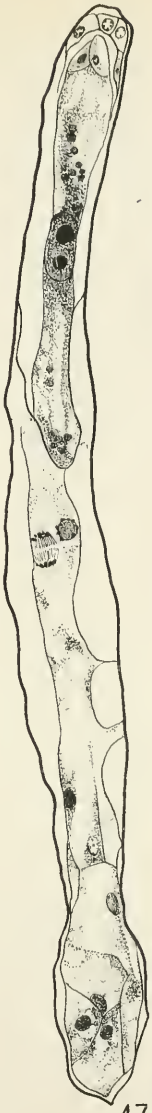
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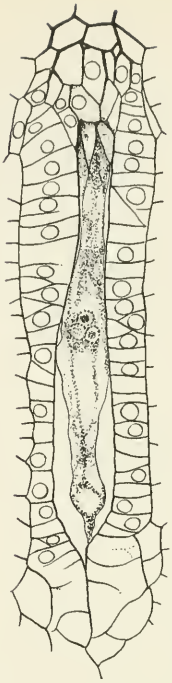
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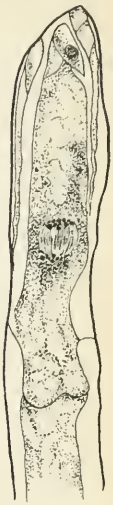
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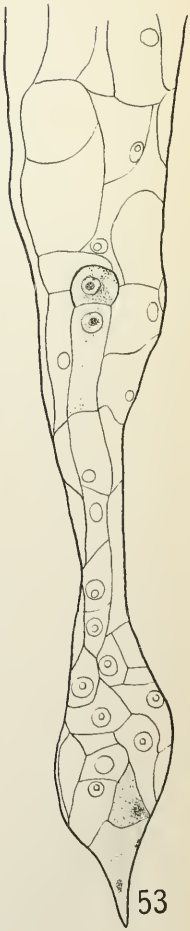
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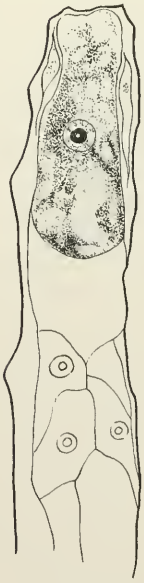
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KANDA on VERBENA

PLATE VII

FIGS. 18–25 magnified 700 diameters; fig. 26 magnified 800 diameters; figs. 22 and 25 are *V. hastata;* all the others *V. angustifolia.*

FIG. 18.—Details of ovule outlined in fig. 15, showing megaspore mother cell.

FIG. 19.—Nucellus of older ovule.

FIGS. 20, 21.—Megaspore mother cell nucleus dividing into two (20), and four (21).

FIG. 22.—Growth of fertile megaspore and its encroachment on sterile cells; nucellus cells somewhat stretched.

FIG. 23.—Embryo sac with 2 nuclei.

FIG. 24.—Embryo sac with 4 nuclei, reconstructed from 4 sections.

FIG. 25.—Embryo sac with polar nuclei in contact.

FIG. 26.—Details of a part of ovary outlined in text fig. 27, showing mature embryo sac invested by jacket; proteid-like substance in space between ovule and carpel.

PLATE VIII

FIGS. 28–41 magnified 700 diameters; figs. 42–45 magnified 800 diameters; figs. 42, 45 are *V. stricta;* all the others *V. angustifolia.*

FIG. 28.—Longitudinal section of young anther showing sporogenous cell row and surrounding layers.

FIGS. 29, 30.—Transverse and longitudinal sections through an older anther, showing granular and mostly binucleate tapetal cells: fig. 29, cells of middle layer, also granular; fig. 30, some rows of pollen mother cells, with nuclei in synapsis.

FIG. 31.—Three pollen mother cells in first division; tapetal cells with 2 nuclei.

FIG. 32.—Two pollen mother cells in anaphase of first division.

FIG. 33.—Early telophase of second division in pollen mother cell.

FIGS. 34, 35.—Tetrad formation; some cytoplasm of mother cell concerned in wall formation.

FIG. 36.—Tetrad formation; cytoplasm of mother cell not concerned in wall formation.

FIGS. 37–41.—Successive stages of development of pollen grain: fig. 39, pollen with starch grains; fig. 40, pollen with large vacuole; fig. 41, pollen with vegetative and generative nuclei.

FIG. 42.—Pollen tube just thrusting itself through nucellar cap.

FIGS. 43, 44.—Fertilization: fig. 43, male nuclei fusing with egg and endosperm nucleus; pollen tube and starch grains shown.

FIG. 45.—First division of primary endosperm nucleus followed by wall formation.

PLATE IX

FIGS. 46–53 magnified 800 diameters; figs. 64–68 magnified 700 diameters; figs. 50, 68 are *V. hastata;* figs. 64, 65, *V. stricta;* figs. 66, 67, intermediate form between *V. stricta* and *V. hastata;* all others are *V. angustifolia.*

FIG. 46.—Embryo sac separated into micropylar and antipodal chambers: nucleus in micropylar chamber just in mitosis; reconstructed from 4 sections.

FIG. 47.—Embryo sac in which endosperm tissue is developing from micropylar end; single large undivided nucleus with 2 nucleoli in antipodal chamber.

FIGS. 48, 49.—Two portions of one embryo sac: fig. 48, antipodal chamber still 1-celled; fig. 49, micropylar chamber filled with tissue.

FIG. 50.—Embryo sac retarded in development by absence of starch in jacket; only 3 nuclei in center.

FIG. 51.—Mitosis of endosperm nucleus in antipodal chamber.

FIGS. 52, 53.—Two parts of more advanced embryo sac: fig. 52, antipodal part with one large resting cell; fig. 53, micropylar part with filamentous embryo.

FIGS. 64, 65.—Pollen mother cell in reduction division: fig. 64, metaphase of first division; fig. 65, early telophase of second division.

FIGS. 66, 67.—Pollen mother cells: fig. 66, metaphase and telophase of first division; fig. 67, telophase of second division.

FIG. 68.—Pollen mother cell in telophase of second division.

A CHEMICAL ANALYSIS OF SUDAN GRASS SEED

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 258

(WITH ONE FIGURE)

F. M. SCHERTZ

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A CHEMICAL ANALYSIS OF SUDAN GRASS SEED

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 258

(WITH ONE FIGURE)

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A CHEMICAL ANALYSIS OF SUDAN GRASS SEED

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 258

F. M. Schertz

(WITH ONE FIGURE)

The method as here outlined was originally taken from the methods of Waldemar Koch,[1] who employed it in the analysis of brain tissues. The method was then further modified by F. C. Koch,[2] of the department of physiological chemistry, University of Chicago, where the work was chiefly on animal tissues. The method was again modified to meet the needs of plant tissues.

Outline of method

Dry or germinating seeds

Soluble portion (F_1+F_2) — Insoluble residue (F_3)

Ether soluble portion (F_1) — Water, alcohol soluble portion (F_2)

Fraction 1 (F_1) is the ether soluble portion; fraction 2 (F_2) is the portion soluble in alcohol or water; and fraction 3 (F_3) is the portion which is insoluble in ether, water, or alcohol. The dry seeds were ground finely before making the extraction, while the germinating seeds were ground in a mortar as finely as possible. The material was then placed in the extraction cups and extracted for 4 hours. A 1-hour extraction with ether was then made and the ether extract was added to the alcohol extract. The residue was dried, ground in a mortar, and then a water extraction was made. This water extraction and the residue was then made up to 70 per cent alcohol and again extracted with 95 per cent alcohol for 12 hours. In some cases this extraction was found to be insufficient,

[1] Koch, Waldemar, Methods for the quantitative chemical analysis of anima tissues. Archives Neurology and Psychiatry 4: 11. 1909; also Jour. Amer. Chem Soc. 31: 1329–1364. 1909.

[2] Outline for the analysis of tissues as prepared by F. C. Koch.

and consequently the extraction was prolonged for another 12 hours or more. The extraction was conducted at the boiling point of the solvent, using the KOCH extractor.

F_1 and F_2.—All of the alcohol, water, and ether extracts were added to each other, and then the whole was rapidly evaporated down to a thick syrup on the water bath. It was then transferred to a vacuum desiccator and dried until nearly a constant weight was obtained. This took from one to three weeks. The air in the desiccator was changed once or twice daily. This gave the weight of F_1 and F_2. The dry mixture of F_1 and F_2 was now extracted with anhydrous ether; this extract was F_1, and the residue was F_2. The evaporating dish plus F_2 was dried and again weighed, giving the weight of F_1 by difference, and also the weight of F_2. The ether extract F_1 was divided into two portions, one portion being used for the determination of sulphur and phosphorus, and the other for nitrogen. The residue was dissolved or suspended in 70 per cent alcohol and made up to a volume of 1000 cc. Of this, 50 cc. was used to determine the total sugars; 100 cc. for ash and for solids; 200 cc. for nitrogen; 100 cc. for free reducing sugars; and 550 cc. for sulphur and phosphorus.

F_3.—F_3 was then dried at 105° C. in an electric oven to nearly a constant weight. The whole was then pulverized thoroughly and fractions of it, ranging from 0.5 to 2.0 gm., were used for the determination of sulphur, phosphorus, nitrogen, total carbohydrates, ash, and crude fiber.

Moisture was obtained by difference. Nitrogen was estimated by means of the Kjeldahl method as modified by Gunning and Arnold. The nitrogen was multiplied by 6.25 to give the protein. Sulphur was estimated by the fusion ($Na_2CO_3+KNO_3$) method, precipitated, and weighed as $BaSO_4$. The filtrate from the sulphur determination was used and the phosphorus was determined from it by the Neumann-Pemberton method, by titration. Organic matter was determined by taking the weights of the ash F_2 and F_3 from the dry weights of F_2 and F_3 respectively. Sugars were estimated by the Bertrand volumetric method in connection with the Munson and Walker tables. Total reducing sugars were found by adding 10 cc. of HCl (sp. gr. 1.125) for every 100 cc.

of water used with the sample, and then boiling on a reflux condenser for 2.5 hours. They were then estimated as glucose.[3] Crude fiber was determined after the method in Bulletin no. 107, Bureau of Chemistry. 1912.

Analysis of unhulled dry seeds

The air dry weight of the seeds used in each case was 25 gm. The seed analyzed was that of Sudan grass (*Holcus halepensis sudanensis* [Piper] Hitchcock or *Andropogon halepensis sudanensis* Piper). In each case two analyses were made and the results, together with the average of these two, are given in table I. The hulled seed was 70.62 per cent of the whole seed by weight, hence the hulls were 29.38 per cent of the whole seed by weight.

Analysis of seeds after germination

An analysis was made of the unhulled seeds which were kept in the refrigerator for 16 days at a temperature ranging from 8 to 20° C. A small percentage of the seeds showed signs of sprouting. In each case 25 gm. of seed were used.

This study was undertaken with the hope of discovering some of the early changes which take place on germination, and also because Sudan grass has promise as a forage grass. In comparing the unhulled dry seeds with the unhulled germinated seeds, it was found that the weight of F_1 remained constant, F_2 lost 2 per cent, and F_3 lost 3 per cent on germination. The protein in F_1 decreased, while that of F_2 increased somewhat. The total protein content of the germinated seeds increased about 1 per cent, due to the building of protein from the reserve substances. No change of importance was noted regarding the sulphur or phosphorus content. The ash of F_2 increased slightly at the expense of the ash of F_3. The amount of organic matter in F_2 decreased 1.5 per cent, while that of F_3 decreased 3 per cent; or a total loss of organic matter of about 5 per cent due to respiration. The greatest changes were found in the sugars. The total reducing sugar of F_2 decreased 2 per cent, free reducing sugar decreased slightly, and the total carbohydrates decreased about 9 per cent. The decrease in sugar-like products

[3] MATHEWS, ALBERT P., Physiological chemistry. 2d ed. New York. 1916.

TABLE I

	I	II	Average
Moisture		14.05	13.95
Weight of F_1		3.69	3.69
" " F_2		9.54	9.54
" " F_3	72.92	72.72	72.82
Total		100.00	100.00
		Protein*	
F_1		0.02	0.02
F_2		1.23	1.23
F_3	5.44	4.96	5.20
Total		6.20	6.45
		Sulphur	
F_1		0.02	0.02
F_2		0.05	0.05
F_3	0.07	0.09	0.08
Total		0.16	0.15
		Phosphorus	
F_1		0.004	0.004
F_2		0.06	0.06
F_3	0.20	0.24	0.22
Total		0.304	0.284
		Ash (inorganic matter)	
F_2		0.65	0.65
F_3	4.61	4.56	4.58
Total		5.21	5.23
		Organic matter	
F_2		8.89	8.89
F_3	68.31	68.16	68.24
Total		77.05	77.13
		Sugars	
F_2 total reducing		2.39	2.39
F_2 free reducing		0.96	0.96
F_3 carbohydrates	58.59	62.36	60.47
Total		64.75	62.86
		Crude fiber	
F_3	5.12	4.77	4.95

*The whole seed was analyzed and it gave a total for protein of 7.23 and 7.38 per cent.

TABLE II

RESULTS OF ANALYSIS OF HULLED DRY SEEDS*

	I	II	III	Average
Moisture	12.53	.....	12.76	12.46
Weight of F_1	4.24	.....	4.72	4.48
" " F_2	6.56	.....	7.87	7.22
" " F_3	76.67	76.19	74.65	75.84
Total	100.00	.....	100.00	100.00
	Proteins			
F_1	.....	.....	0.01	0.01
F_2	1.06	.....	1.07	1.06
F_3	7.28	7.26	7.54	7.36
Total	.....	.....	8.62	8.43
	Sulphur			
F_1	0.01	.....	0.01	0.01
F_2	0.05	.....	0.08	0.06
F_3	0.27	0.21	0.17	0.22
Total	0.33	.....	0.26	0.29
	Phosphorus			
F_1	.....	.....	0.002	0.002
F_2	0.08	.....	0.10	0.09
F_3	0.26	0.22	0.25	0.24
Total	.....	.....	0.352	0.332
	Ash			
F_2	0.82	.....	0.82	0.82
F_3	1.41	1.36	1.26	1.34
Total	2.23	.....	2.08	2.16
	Organic matter			
F_2	5.74	.....	7.05	6.40
F_3	75.26	74.83	73.39	74.50
Total	81.00	.....	80.44	80.90
	Sugars			
F_2 total reducing	0.34	.....	.....	0.34
F_2 free reducing	0.27	.....	0.26	0.27
F_3 carbohydrates	67.29	64.72	66.48	66.16
Total	67.63	.....	.....	66.50
	Crude fiber			
F_3	1.08	0.98	.....	1.03

* Air dry weight of the seeds used in each case was 25 gm.

TABLE III

	I	II	Average
Moisture absorbed	48.22	45.72	46.97
Moisture	18.34	19.12	18.73
Weight of F_1	3.25	3.94	3.60
" " F_2	8.03	7.58	7.80
" " F_3	70.38	69.36	69.87
Total	100.00	100.00	100.00
	Proteins		
F_1	0.01	0.01	0.01
F_2	2.20	2.66	2.43
F_3	5.10	4.93	5.02
Total	7.31	7.60	7.46
	Sulphur		
F_1	.....	0.01	0.01
F_2	0.05	0.05	0.05
F_3	0.16	0.13	0.15
Total	.....	0.19	0.21
	Phosphorus		
F_1	0.002	0.001	0.001
F_2	0.06	0.08	0.07
F_3	0.21	0.23	0.22
Total	0.272	0.311	0.291
	Ash		
F_2	0.85	.....	0.85
F_3	4.54	4.49	4.52
Total	5.39	.....	5.36
	Organic matter		
F_2	7.19	.....	7.19
F_3	65.84	64.87	65.36
Total	73.03	.....	72.55
	Sugars		
F_2 total reducing	.....	0.49	0.49
F_2 free reducing	0.40	0.33	0.37
F_3 carbohydrates	48.84	53.24	51.04
Total	.....	53.73	51.53
	Crude fiber		
F_3	5.54	4.63	5.08

was about 11.5 per cent, due to respiration. Crude fiber remained practically constant.

When the hulled dry seeds were compared with the unhulled dry seeds, it was found that the weight of F_1 was 1 per cent greater in the former, and it was 2 per cent greater in the latter for F_2, while F_3 of the former was about 3 per cent greater. The proteins of

TABLE IV

UNHULLED DRY SEEDS

Material	I	II	Average
Free reducing sugars	1.10	0.93	1.02
Sucrose-like sugars	1.94	2.43	2.19
Total reducing sugars	3.32	2.71	3.02
Total carbohydrate F_3	60.00	59.60	59.80
Total	63.32	62.31	62.82*
	Unhulled seed grown at room temperature		
Free reducing sugars	1.02	0.96	0.99
Sucrose-like sugars	1.54	1.39	1.47
Total reducing sugars	3.24	3.25	3.24
Total carbohydrate F_3	43.36	45.88	44.62
Total	46.60	49.13	47.86
	Unhulled seed grown in refrigerator		
Free reducing sugars	0.89	0.75	0.82
Sucrose-like sugars	1.64	1.34	1.49
Total reducing sugars	2.69	2.69	2.69
Total carbohydrates F_3	43.51	43.93	43.72
Total	46.20	46.62	46.41

* Ten gm. of seed were hydrolyzed for 2.5 hours and gave a total carbohydrate of 65.30 per cent

F_1 and F_2 were about the same, but the protein of F_3 of the hulled dry seeds was more than 2 per cent greater. The ash of F_2 was slightly more in the hulled dry seeds, while the ash of F_3 was over 3 per cent greater in the unhulled seeds; hence a greater part of the ash was in the hulls. The organic matter of F_2 of the unhulled dry seeds was 2.5 per cent greater, while in F_3 it was 6 per cent less. The free reducing sugars were slightly greater in the unhulled seeds,

the total reducing sugars were 2 per cent greater, while the carbohydrates were over 6 per cent less. Five times as much crude fiber was found in the unhulled seeds.

A further analysis of the sugars was then made. Two samples of 25 gm. each of the dry seed were analyzed for sugars alone. Two samples of 25 gm. each were grown at room temperature (16–24° C.) for 3 days, and two other samples were grown in the refrigerator for 32 days. The seeds in each case were extracted as indicated into the two portions F_2 and F_3. F_2 was then evaporated down and made up to a volume of 500 cc., of which 100 cc. was used for the determination of total reducing sugars; three 50 cc. samples for the inversion of cane sugar by weak hydrolysis at 67–69° C. for 10 minutes; and the remainder was used for free reducing sugars. All of the F_3 was hydrolyzed for 2.5 hours by adding 300 cc. water and 30 cc. hydrochloric acid (sp. gr. 1.125). From small portions of this the total sugars of F_3 were determined.

From table IV it is seen that when the seeds germinate the sucrose-like sugars decreased about 1 per cent, while there was a decrease in the total carbohydrates of about 15 per cent.

TABLE V

SUDAN GRASS COMPARED WITH OTHER SEEDS

Seeds	Water	Protein	Fat	N-free extract	Crude fiber	Ash	Sugar
Triticum sativum	13.37	10.93	1.65	70.01	2.12	1.92	2–7
Hordeum sativum	12.95	10.01	1.87	67.88	4.23	3.06	6–7
Secale cereale	13.37	11.19	1.68	69.36	2.16	2.24	2–3
Zea Mays	13.32	9–10	4–5	68–69	1.6–2.7	1.60	1.5–3.
Sorghum saccharatum	14.58	9.44	3.18	68.55	2.54	1.71	.....
Oryza sativa hulled	13.17	8.13	1.27	75.50	0.88	1.03	1–2
Oryza sativa unhulled	.....	.....	2.00	.....	.....	3.57	.....
Avena sativa	12.8	10.25	5.27	59.68	9.97	3.02	2–7 5
Holcus halepensis sudanensis unhulled	13.94	6.44	3.69	75.74*	4.95	5.24	2.39†
Holcus halepensis sudanensis hulled	12.47	8.43	4.48	71.43*	1.03	2.16	3.44†
Unhulled germinated seed in refrigerator	18.72	7.46	3.60	59.78*	5.08	5.36	2.69†
Sudan grass seed (Kansas)‡	10.47	13.69	3.81	63.63	5.38	3.09	.....

* 100 − (protein + ether extract + ash + moisture + crude fiber).

† Total reducing sugars as dextrose.

‡ THOMPSON, G. E., Sudan grass in Kansas. Kansas Agric. Exper. Sta. Bull. 212. 1916.

It is of interest to compare these results with those of some other workers. KJELDAHL, working on barley seed, found about 4.7 per cent cane sugar in the green malt and 1.1 per cent in the ungerminated barley. O'SULLIVAN found in ungerminated barley 0.8–1.6

and in malt 2.8–6.0 per cent cane sugar. These results on Sudan grass gave in each case less than 1.0 per cent of cane sugar, figuring the reducing sugar as cane sugar.

Compared with other grasses[4] it is very similar to *Sorghum avenaceum*, which gave the following results: ash 5.63, protein 3.29, cellulose 36.7, and fat 1.67 per cent. Of the ash, 1.5–3.0 per cent was CaO, P_2O_5, MgO, and SO_3.

Catalase activity

In each case 0.2 gm. (dry weight) of the seed was used. The results are given in cubic centimeters of oxygen set free in 10 minutes at 20° C.

Dry seeds		Seeds at room temperature 3 days
Hulled seeds	Unhulled seeds	Unhulled seeds
13.8	15.5	54.0
17.2	17.0	65.2
		68.2
15.0	16.25	
		62.4

Seeds in refrigerator 31 days	
Unhulled seeds	Unhulled seeds
45.0	46.0
50.8	50.6
45.6	50.0
47.1	49.0

The seeds which were grown in the refrigerator showed less catalase activity; part of this lessened activity may be due to the lowered temperature, but part of it undoubtedly was also due to the fact that the seeds at room temperature had grown slightly more than those in the refrigerator.

Microchemistry

A brief microchemical analysis was undertaken in order to locate the materials in the tissue of the seed, as well as to get an idea of how much was present (fig. 1).

Practically all of the cell walls gave the blue color reaction with 75 per cent H_2SO_4 and iodine, except the two small regions of the integument at each end of the caryopsis. With phloroglucin-HCl a cherry red color was observed in the pericarp integument near

[4] Wehmer, C., Die Pflanzenstoffe. Jena. 1911.

the micropylar end of the caryopsis. With acetone and a drop of concentrated HCl a red color was noted on the pedicel, and especially was the red prominent in the whole pericarp integument. This indicated strongly the presence of methyl pentosan, and perhaps araban and xylan. No callose was observed in any of the tissues. With ruthenium red, the pericarp integument and the cell membranes of the starchy endosperm gave slight tests, while the scutellum, plumule, plumule sheath, radicle, and root shoot gave a strong reaction, indicating the presence of much pectic substance. Small particles in the cells also gave a pectose reaction. The phloroglucin-HCl tests showed only traces of lignin, if any, present in the pedicel and in the glume. Upon heating the tissues with concentrated HNO_3 and concentrated $KClO_3$, ceric acid was observed to issue from the tissues of the pericarp integument. Suberin was present here.

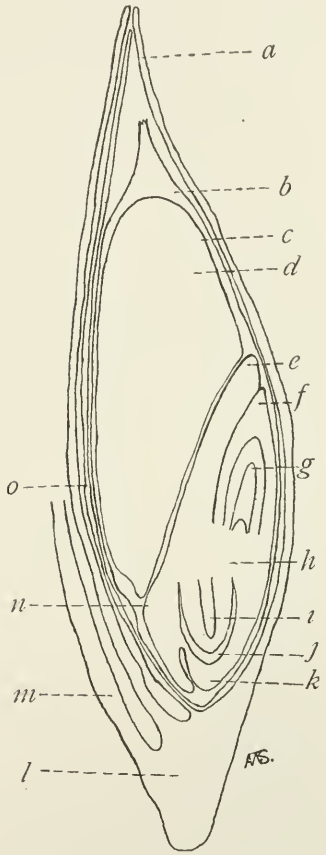

FIG. 1.—Longitudinal section of grain of Sudan grass: *a*, glume; *b*, pericarp; *c*, aleurone layer; *d*, endosperm; *e*, scutellum; *f*, coleoptile; *g*, plumule; *h*, embryo node; *i*, radicle; *j*, root cap; *k*, coleorhiza; *l*, pedicel; *m*, basal seta; *n*, glandular layer of scutellum; *o*, lodicule.

All cells of the embryo, and especially the cells of the embryo at the micropylar end, were rich in oil. The fat-containing cells of the endosperm stained heavily with Sudan III. Also, the epithelial layer had some fat present. The whole of the embryo became red when treated with concentrated H_2SO_4, and later took a greenish hue. Hence, phytosterol was thought to be present in the embryo, and also in a portion of the seed coat at the micropylar end of the caryopsis.

Silicon was found in the pericarp, as was shown by heating a dry section of the tissues with phenol. Tannins were found in the glumes and in the outer coats of the seeds, where red and purplish colors were observed, which were probably due to the oxidized tannins.

Two sizes of starch grains were found. The endosperm cells were filled with large sized starch grains, while the pericarp integument, the pedicel, and the basal seta had smaller grains in them.

Neither dextrin nor glucose was present in the embryo or in the endosperm, but considerable was present in the hulls. Amylodextrin was found in all of the endosperm cells in rather large quantities. The layers of the cells of the caryopsis outside of the fat-containing endosperm cells all gave a positive reaction for glucose when treated with copper tartrate and sodium hydroxide.

TABLE VI

MICROCHEMISTRY OF SUDAN GRASS SEED

Part of seed	Cellulose	Pentoses	Pectic substances	Lignin	Suberin	Fat	Phytosterol	Tannin	Starch	Glucose	Amylodextrin
Pedicel	+*	+		+					+		
Glume	+			+				+		++	
Basal seta									+		
Lodicule	+										
Pericarp	+	++	+		+		+	+	+	+	
Aleurone layer						++					
Endosperm	+		+						++		++
Epithelial layer						+					
Scutellum	+		++			+	+				
Embryo node	+		++			+	+				
Radicle	+		++			++	+				
Coleorhiza	+		++			++	+				
Plumule	+		++			+	+				
Coleoptile	+		++			+	+				

* + = present; ++ = present in large amount.

In conclusion, I wish to acknowledge my obligations to Professor WILLIAM CROCKER, under whom this work was done, for his advice and valuable criticisms; to Dr. S. H. ECKERSON for her untiring interest and advice relative to the microchemistry; and to Professor F. C. KOCH for his helpful suggestions in the methods of chemical determinations involved.

BUREAU OF PLANT INDUSTRY
WASHINGTON, D.C.

FORMATIVE EFFECT OF HIGH AND LOW TEMPERATURES UPON GROWTH OF BARLEY: A CHEMICAL CORRELATION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 259

(WITH EIGHTEEN FIGURES)

H. L. WALSTER

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Introduction

Cereals are commonly considered cool temperature crops. Cool seasons are known to favor cereal production, warm seasons to hinder cereal production. Physiologists have correlated these observations with the general effects of temperature upon the growth and maturation of the crop, but have given little attention to possible effects of the initial germination temperature upon the subsequent course of development of the plant. The investigation here reported is a study of the effects of high and low temperatures and concomitant variations in the supply of nitrogen, phosphorus, and potassium respectively upon the course of development of the barley plant. A chemical correlation has been established between temperature and nutrition effects.

Literature

ADERHOLD (1), working with young kohlrabi plants, noted that exposures of the young plants to temperatures of $-2°$ C. to $-8°$ C. for 8–10 hours tended to cause the plant to shoot into flowering instead of forming the desired "ball."

GUTZEIT (5) repeated ADERHOLD'S work and found by a rather extensive set of experiments that exposures to temperatures below zero had no effect on stem or shoot production in kohlrabi, beets, or various other plants. He did find, however, that a period at +4° C. during germination and early growth caused about 30 per cent of certain beets to produce shoots very early the first year. Some of the shoots produced only very short stems, and the plants were otherwise normal, while other shoots grew continuously and early produced flowers and seeds. Beets of exactly the same kind when kept at +22° C. during germination and early growth showed no shoot production the first year. Only such beets as were predisposed to early shoot production could be thus forced by low temperatures, so hereditary characters as well as temperature enter in as determining factors. GUTZEIT suggests that this temperature response explains why early seeding of beets causes much premature shoot production, whereas late seeding gives little or none. On the basis of other experiments conducted by himself, as well as data from the literature, GUTZEIT concludes that low temperatures during germination and early growth favor stem formation, while high temperatures at this time inhibit stem formation.

APPEL and GASSNER (2) noted in the experimental fields of summer cereals at the Agricultural Experiment Station at Dahlem, Germany, a peculiar sickness, the plants becoming light green, and the older leaves turning yellow. Since neither animal nor plant pests seemed to be attacking the cereals, an explanation for their condition was sought in unfavorable soil and weather relations. Greenhouse experiments conducted by APPEL and GASSNER led them to attribute the peculiar conditions of these summer cereals to a too high germination temperature.

They grew barley in pots in the greenhouse, keeping one lot at 20–25° C. and the other lot at 5–7° C. When the plants at the higher temperature had reached a height of 15 cm., those in the cool house had just come up. Both sets were then transferred to the open and kept under like conditions. After three weeks the barley plants from the warm house began to show signs of injury, the older leaves yellowing at their tips, and only the youngest leaves remaining green. The barley plants from the cool house

soon outstripped the high temperature plants, finally reaching twice the size. Figures of APPEL and GASSNER's plants show that there was an excessive leaf production and little stem production at the higher temperature. These investigations suggested that the light color of the leaves was due to nitrogen hunger, but they were unable to get any beneficial results from nitrogen fertilization. The addition of iron salts also had no favorable effect.

GASSNER (3) has made extensive observations and experimental studies upon the growth and development of cereals in subtropical climates, the experiments being carried out in the phytopathological experimental fields of the University of Montevideo, Uruguay. In considering the choice of varieties of summer cereals suitable for cultivation in Uruguay, he emphasizes the importance of temperature in the early stages of development, and suggests that decreased yields are often due to the lack of the necessary cold requirements (Kälteansprüche) in the early stages of growth. GASSNER quotes HELLRIEGEL (6) on the temperature relations of small 4-rowed barley. HELLRIEGEL maintained that in the first half of the vegetative period of the barley, the period of leaf and culm formation, an average daily temperature of about 15° C. is the best, whereas in the second half of the vegetative period, the period of head development and grain formation, a temperature of 17–18° C. is the most favorable. HELLRIEGEL therefore insists upon two different temperature optima in development of barley, the line of demarcation between the two optima being placed at the time of shooting.

GASSNER summarizes his views as follows (translated from the original article):

> We can therefore say that for winter cereals, as well as for summer cereals, the yield of a given variety of a cereal in a given climate is among other things dependent upon the influence of the climatic factors in the first stage of development in such a way that varieties of high "cold requirements" in their youth require a colder climate than varieties with lower "cold requirements," and that incomplete fulfillment of these requirements causes bad development and depression of the yield.

GASSNER states that the death and yellowing of the leaves of young plants previously described by APPEL and GASSNER rarely

occurs in Uruguay. He notes, however, that the culm habit in Uruguayan oats and rye germinated at high temperatures is distinctly recumbent, whereas it is upright from the beginning in the case of plants grown from seeds germinated at low temperatures.

he low temperature plants begin the formation of the culm (shooting) much earlier than do the high temperature plants. A typical experiment with oats is outlined as follows:

Date of seeding	Temperature during germination	Date of transfer into field	Beginning shooting
January 18	January 18–23, 6–9°; January 23–25, 25°	January 25	March 15
January 18	January 18–20, 25°; January 20–25, 6–9°	January 23	No shoot formation on April 25, shooting not expected before October

In another series it was found that even 24 hours of exposure to a germination temperature of 25° led to the same abnormal course of development as indicated in the second series here quoted.

Gassner and Grimme (4) have made one attempt to correlate the effects of germination temperatures and the resistance of cereals to frost injury. They analyzed the first leaves of winter and spring rye germinated at 5–6° C. and at 28°. They found that the seedlings germinated at the lower temperature had a higher sugar content than seedlings germinated at the high temperature; moreover, seedlings of a hardy winter rye had a higher sugar content than those of a spring rye grown under the same conditions. Their results with rye are shown in table I.

Hutcheson and Quantz (7) conducted experiments on the effect of greenhouse temperatures on the growth of the small grains: wheat, oats, barley, and rye. All four crops were grown under four temperature conditions, namely, 14.4° C., 16.6° C., 18.3° C., and 23.9° C. The higher temperature range had a distinctly detrimental effect upon the growth of the barley and a less harmful effect upon the growth of wheat and rye, while oats had a normal course of development at all the temperatures used, although the oat culms were weaker at the higher temperatures. The high

temperature barley plants showed an excessive development of tillers and no indication of ever heading. Inspection of the figures shows that the leaves of the high temperature plants were abnormally long, and especially so in the case of the barley. The general growth characters obtained by HUTCHESON and QUANTZ were obtained in the present investigation in the case of high temperature, high nitrogen series (fig. 13). These authors grew the grain

TABLE I

SUGAR CONTENT OF FIRST LEAVES OF RYE* (PERCENTAGE OF DRY WEIGHT)

SERIES NO.	TOTAL SUGAR	GERMINATION TEMPERATURE 5–6° C.		GERMINATION TEMPERATURE 28° C.		
		Reducing sugar	Non-reducing sugar	Total sugar	Reducing sugar	Non-reducing sugar
	Petkuser winter rye					
I	42.19	34.93	7.26	40.92	32.56	8.36
II	43.14	35.86	7.28	39.79	31.14	8.65
III	41.92	34.84	7.08	39.13	31.08	8.05
IV	42.31	35.85	6.46	40.73	33.94	6.79
V	40.97	32.31	8.66	39.52	34.11	5.41
	Petkuser spring rye					
I	36.58	29.41	7.17	31.57	27.13	4.44
II	37.08	30.57	6.51	33.26	26.58	4.68
III	35.39	30.41	4.98	32.59	26.81	5.78
IV	37.65	31.02	6.63	34.56	30.38	4.18
V	35.85	30.21	5.64	32.94	28.16	4.78

* Similar results were obtained with barley.

in 4-inch clay pots, two plants to the pot. No mention is made concerning the substrate used in their experiments.

This investigation of the influence of high and low temperatures upon the growth of barley was planned to ascertain in particular the influence of variations in the supply of nutrient salts with concomitant variations in the temperature. The nutrients varied were nitrogen, potassium, and phosphorus. Chemical analyses were made in order to relate certain observed differences in growth to possible differences in the chemical composition.

Method

Culture solutions

The method of sand culture was used throughout these experiments, the sand used being a highly pure Ottawa silica sand obtained from Ottawa, Illinois. Two gallon glazed stone jars were used as the culture vessels, each jar receiving 11.4 kilos of sand. The water content of each jar was maintained at approximately 13 per cent of the dry weight of the sand by means of frequent weighing. Tottingham's culture solution was used in diluted form. This solution has the following composition:

Solution A: KNO_3—0.0034 M (0.3437 gm. per liter)
KH_2PO_4—0.0108 M (1.4692 gm. per liter)
$MgSO_4$—0.0081 M (0.9750 gm. per liter)

Solution B: $Ca(NO_3)_2$—0.0101 M (1.6573 gm. per liter)

Enough of these salts to make 100 liters of culture solution were dissolved and made up to 2 liters, the $Ca(NO_3)_2$ being made up in a separate 2-liter portion in order to prevent precipitation of insoluble calcium salts in the highly concentrated solution. The mixture of these two solutions was designated solution A B, and 7.5 cc. of each of these solutions were added to 1500 cc. of distilled water for the initial dose of nutrient solution. This quantity of nutrient solution was applied to the jars designated in the outline of the scheme of the experiment at the time of planting (March 1). In addition, 0.01 gm. of $FeCl_3$ was added to each culture one week after sowing. On April 4 each A B culture received a second dose of 7.5 cc. of this normal nutrient solution. All cultures receiving only A B solutions will be referred to hereafter as "normal."

Solutions lacking in P, N, and K were also made. The amount of salts indicated in the respective tables were dissolved and made up to 2000 cc. with distilled water; 75 cc. of these solutions made up to 1500 cc. were used as initial doses. Similarly, solutions were made up in which the P, N, and K were supplied in one-fourth the concentration of that found in solution A B.

Solution C
(lacking in phosphorus)

3.437 gm. KNO_3
8.8561 gm. KCl
23.855 gm. $Ca(NO_3)_2$, $4H_2O$
19.789 gm. $MgSO_4$, $7H_2O$

Solution D
(phosphorus in one-fourth concentration)

3.437 gm. KNO_3
3.873 gm. KH_2PO_4
6.642 gm. KCl
23.855 gm. $Ca(NO_3)_2$, $4H_2O$
19.789 gm. $MgSO_4$, $7H_2O$

Solution E
(lacking nitrogen)

2.723 gm. KCl
14.6923 gm. KH_2PO_4
12.353 gm. $CaCl_2$, $2H_2O$
19.789 gm. $MgSO_4$, $7H_2O$

Solution F
(nitrogen in one-fourth concentration)

0.8592 gm. KNO_3
2.0422 gm. KCl
14.6923 gm. KH_2PO_4
19.7890 gm. $MgSO_4$, $7H_2O$
4.1432 gm. $Ca(NO_3)_2$, $4H_2O$
9.264 gm. $CaCl_2$, $2H_2O$

Solution G
(lacking potassium)

2.8894 gm. $NaNO_3$
17.0692 gm. Na_2HPO_4, $12H_2O$
23.855 gm. $Ca(NO_3)_2$, $4H_2O$
19.789 gm. $MgSO_4$, $7H_2O$

Solution H
(potassium in one-fourth concentration)

0.8592 gm. KNO_3
2.1671 gm. $NaNO_3$
3.873 gm. KH_2PO_4
12.8019 gm. Na_2HPO_4, $12H_2O$
19.789 gm. $MgSO_4$, $7H_2O$
23.8558 gm. $Ca(NO_3)_2$, $4H_2O$

To certain of the A B cultures extra doses of N, K, and P, alone, and in all possible combinations, were added in the amounts and at the times indicated in the schematic outline. These extra doses were supplied in the form of solutions of $NaNO_3$, KCl, and Na_2HPO_4, $12H_2O$ respectively. All cultures were run in triplicate. Certain of the replicates in each set of triplicates received a modified supplementary treatment, as indicated in table II, the letters N, K, and P indicating $NaNO_3$, KCl, and Na_2HPO_4, $12H_2O$ respectively.

Oderbrucker barley (Wisconsin No. 5) was seeded March 1. About 30 seeds were sown per culture, the cultures being thinned to 25 plants per culture. This heavy seeding was purposely chosen in order to prevent tillering, so that the course of development of a plant with a single culm could be followed.

Temperature and humidity control.—The temperature of the greenhouses was controlled by means of automatic thermoregulators. The lower temperature selected was 15° C., the higher temperature 20° C. The degree of control obtained is shown in

TABLE II

OUTLINE OF GREENHOUSE EXPERIMENTS

Jar no.*	General treatment	Supplementary treatment
1, 2, 3, 64, 65, 66	Distilled water only	.....
4, 5, 68, 69	Solution C	.....
6, 67	Solution C	2 gm. P added April 27
7, 8, 9, 70, 71, 72	Solution D	1 gm. N added April 26
10, 11, 12, 73, 74, 75	Solution A B	1 gm. N added April 26
13, 14, 15, 76, 77, 78	Solution A B+1 gm. P	Second dose of 1 gm. of P added March 30
16, 17, 80, 81	Solution E	.....
18, 71	Solution E	4 gm. N added April 27
19, 21, 82, 84	Solution F	.....
20, 83	Solution F	4 gm. N added April 27
22, 23, 85, 86	Solution A B+1 gm. N	Second dose of 1 gm. of N on March 20; third dose of 2 gm. on April 26; fourth dose of 2 gm. on April 29
24, 87	Solution A B+1 gm. N	Second dose of 1 gm. of N on March 30
25, 26, 27, 88, 89, 90	Solution A B	1 gm. N added April 26
28, 30, 91, 92	Solution G	1 gm. N added April 27
29, 93	Solution G	1 gm. N added April 27; 2 gm. K added April 27
31, 32, 33, 94, 95, 96	Solution H	1 gm. N added April 27
34, 35, 36, 97, 98, 99	Solution A B	1 gm. N added April 27
37, 38, 39, 100, 101, 102	Solution A B+1 gm. KCl	Second dose of 1 gm. K March 30; 1 gm. N April 26
40, 42, 103, 104	Solution A B+1 gm. N, 1 gm. P	Second dose of 1 gm. N and 1 gm. P March 30; third dose of 2 gm. of each April 26; 2 gm. N only April 29
41, 105	Solution A B+1 gm. N, 1 gm. P	Second dose of 1 gm. N and 1 gm. P March 30; 1 gm. N and 2 gm. P April 26
43, 45, 106, 107	Solution A B+1 gm. N	Second dose of 1 gm. N March 30; third dose of 1 gm. N April 26
44, 108	Solution A B+1 gm. N	Second dose of 1 gm. N April 26
46, 47, 48, 109, 110, 111	Solution A B+1 gm. P, 1 gm. K	Second dose of 1 gm. of P and 1 gm. K March 30; 1 gm. N April 26
49, 50	Solution A B+1 gm. N	Second dose of 1 gm. N and 1 gm. K March 30; third dose of 2 gm. of each April 26; 2 gm. more of N April 29

TABLE II—*Continued*

Jar no.	General treatment	Supplementary treatment
51, 114	Solution A B+1 gm. N, 1 gm. K	Second dose of 1 gm. N and 1 gm. K March 30; 1 gm. N and 2 gm. K April 26
52, 53, 54, 115, 116, 117	Solution A B+1 gm. P	Second dose of 1 gm. P March 30; 1 gm. N April 26
55, 56, 119, 120	Solution A B+1 gm. N, 1 gm. K, 1 gm. P	Second dose of 1 gm. each of N, K, and P March 30; of 2 gm. each April 26; 2 gm. N April 29
57, 118	Solution A B+1 gm. N, 1 gm. K, 1 gm. P	Second dose of 1 gm. each of N, K, and P March 30; 1 gm. N and 2 gm. each of K and P April 26
58, 59, 122, 123	Solution A B+1 gm. N, 1 gm. P	Second dose of 1 gm. each of N and P March 30; 2 gm. more of each April 26; 2 gm. of N April 29
60, 121	Solution A B+1 gm. N, 1 gm. P	Second dose of 1 gm. each of N and P March 30; 1 gm. N and 2 gm. P April 26
61, 62, 124, 125	Solution A B+2 gm. P, 1 gm. N	Second dose of 2 gm. P and 1 gm. N March 30; 4 gm. P and 2 gm. N April 26; 2 gm. N April 29
63, 126	Solution A B+2 gm. P, 1 gm. N	Second dose of 2 gm. P and 1 gm. N March 30; 4 gm. P and 1 gm. N April 26

* Jars nos. 1–63 inclusive kept in warm greenhouse; jars nos. 64–126 inclusive kept in cool greenhouse

the thermograph records obtained in the two houses (figs. 1, 2). It will be noted that there was a fairly satisfactory degree of control up to about the middle of April, at which time (April 19) the samples for chemical analyses were taken. The principal fluctuations came at about noon; a considerable temperature difference always existed.

The degree of humidity was not under a complete control as desired, the evaporation rate averaging somewhat higher in the warm house. It is possible that some of the differences noted in chemical composition are due to the higher evaporating power of the air in the warm house. This higher evaporation rate was, of course, a function of the higher temperature.

Observations on growth of barley cultures

During the first two weeks of growth the plants in the warm house, which were several inches high before the plants in the cool house had come up, maintained a more rapid growth rate. The first leaves of all of the plants in the warm house, except those receiving little or no nitrogen, tended to lop over. The low

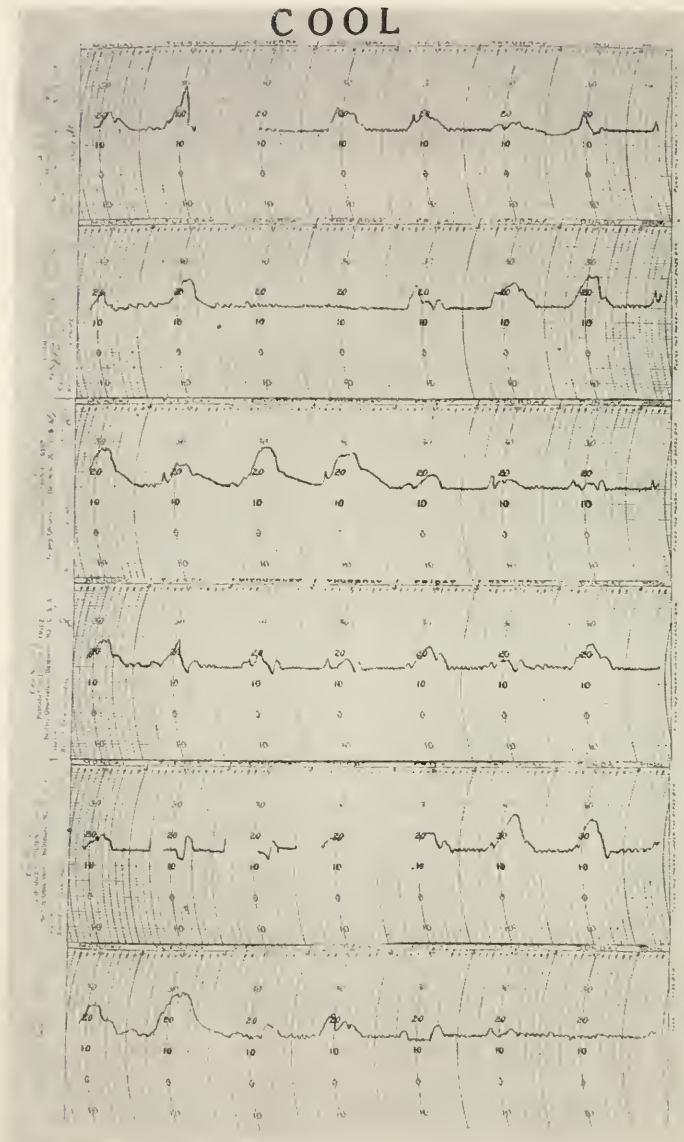

FIG. 1.—Thermograph records showing air temperature in cool house from planting to time of sampling for chemical analysis

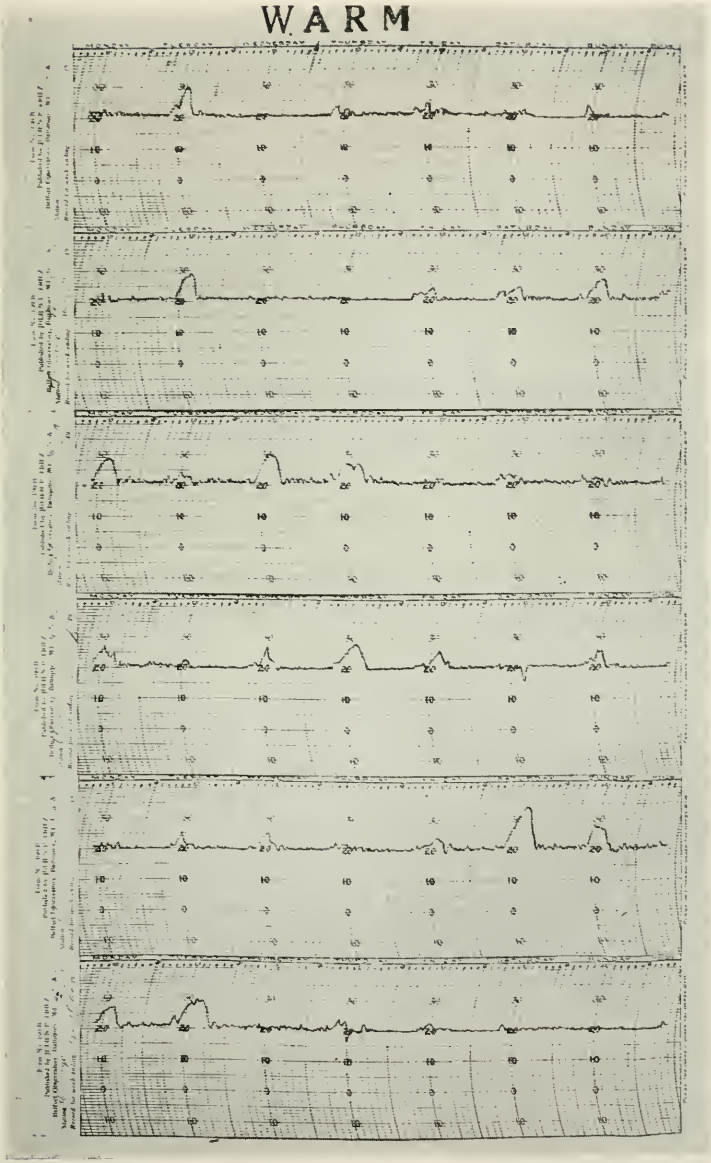

FIG. 2.—Thermograph records showing air temperature in warm house from planting to time of sampling for chemical analysis

nitrogen leaves were in every case stiff and upright. By March 16 the "no phosphorus" series began to show the effect of the deficiency. The "no potassium" series in the warm house showed the

TABLE III

PROPORTION OF LEAVES (BLADE AND SHEATH) AND STEMS IN 100 PARTS OF TOTAL PLANTS, BASED ON GREEN WEIGHT

Culture no. and treatment	Leaves (blades and sheaths) Percentage	Stems Percentage
44. High N warm	92.95	7.05
24. High N warm	88.23	11.73
40. High P and N warm	96.87	3.23
108. High N cool	69.20	30.80
87. High N cool	65.68	34.32
104. High P and N cool	71.37	28.63

greatest lopping over on March 16. About April 1 the plants in the cool house began to outstrip the plants in the warm house in their growth rate, and in particular in their tendency to maintain an upright growth habit. The total amount of tissue formed at

FIG. 3.—Nitrogen series, cool house: note vigorous upright condition of no. 85 as compared with sprawling condition of no. 23 (fig. 4).

the higher temperature was about the same, but it was differently distributed, as will be apparent from the data given in table III.

By April 19 all plants in the cool house had outstripped those in the warm house. The most striking difference between the two

houses was the sprawling condition of the high nitrogen cultures in the warm house, in contrast with their upright condition in the cool house. Figs. 3–15, taken April 24, show the condition of the barley on that date.

FIG. 4.—Nitrogen series, warm house

FIG. 5.—Phosphorus series, cool house: N and K treatment of nos. 68, 71, 75, and 78 "normal" (same as no. 89 in fig. 3).

Figs. 16–18, taken May 16, show the failure of the high nitrogen-high temperature plants to mature normally. Such shooting as was obtained at the higher temperature was due, in the opinion of the writer, to inability to control the moisture supply, because of very great fluctuation in the temperature as the spring season advanced. The writer believes that had it been possible to control absolutely

temperature and moisture supply, the high nitrogen-high temperature series could have been maintained in practically continuous vegetation without any tendency to reproduce. The reason for this belief was the failure of this series to produce any stem (culm) until the water supply fell below the normal previously maintained.

Chemical examination of tissues

In order to ascertain, if possible, the character of the internal processes that determine this very striking formative effect of the

FIG. 6.—Phosphorus series, warm house: N and K treatment of nos. 6, 8, 12, and 15 "normal" (same as no. 25 in fig. 4).

higher temperature in the presence of high nitrogen supply, tissue analyses were made on the leaves (blades plus sheaths) from 100 gm. of total plants from cultures nos. 44, 24, 108, 87, and 104. The plants were selected so that the sample equaled 100 gm. Table III shows the very low percentage of stem material at the higher temperature. Since both leaf-blade and leaf-sheath are active organs in cereals, both were included. The second column in the table shows the green weight in grams of this leaf tissue. The first column in table VI shows the date and hour of taking these samples.

Methods of tissue analysis

The green samples were weighed and immediately preserved by adding enough ethyl alcohol to make a 75 per cent alcoholic solution, and then boiled to arrest enzymic activity. The preserved

Fig. 7.—Potassium series, cool house: N and P treatment of nos. 92, 94, and 100 same as no. 89 in fig. 3; note sprawling condition of "no potash" culture.

Fig. 8.—Potassium series, warm house: N and P treatment of nos. 29, 32, and 38 same as no. 25 in fig. 4.

material was then subjected to the method of tissue analysis devised by Waldemar Koch, and modified by F. C. Koch (8). The method used consisted essentially of 4 hours' extraction with hot ethyl alcohol in a continuous extractor, followed by 1 hour's

extraction with ether, then treatment of the finely ground material with hot water several times, after which the aqueous mixture was made up to a concentration of 75 per cent alcohol and filtered. The insoluble material was then subjected to further extraction with hot alcohol for 24 hours.

The combined extractions were evaporated to dryness on a steam bath, then repeatedly evaporated with absolute ethyl alcohol in order to remove water. The dry hard residue was then

FIG. 9.—Effect of heavy N fertilization: no. 12, normal N (warm house); no. 85, heavy N (cool house); no. 22, heavy N (warm house); no. 75, normal N (cool house).

extracted with anhydrous ether by grinding with a pestle with successive portions of fresh ether. The ethereal extracts were made up to 250 cc., and then divided into suitable aliquots for chemical and dry weight determinations (50 cc. portions). This extraction was designated as fraction 1 (F_1). The ether-insoluble residue was taken up in about 65 per cent alcohol and made up to a volume of 500 cc., 50 cc. portions being taken as aliquots for analysis and dry weight determinations. This was designated as fraction 2 (F_2). Moisture determinations were made on duplicate F_1 and F_2 aliquots

by evaporating almost to dryness on the steam bath and then taking down to constant weight in a vacuum desiccator.

TABLE IV

EFFECT OF TEMPERATURE UPON AMOUNT AND PERCENTAGE OF DRY MATTER AND WATER IN BARLEY LEAVES

Culture no. and treatment	Green weight (gm.)	Dry weight (gm.)	Weight of water (gm.)	Percentage of water	Percentage of dry matter
44. High N warm.....	92.95	12.67	80.28	86.36	13.64
24. High N warm.....	88.23	12.79	75.44	85.50	14.50
41. High P and N warm...	96.87	11.10	85.77	89.47	10.53
108. High N cool.....	69.20	10.92	58.28	84.21	15.79
87. High N cool.....	65.68	10.23	55.45	84.42	15.58
104. High P and N cool....	71.37	11.04	60.23	84.39	15.61

Material insoluble in ether, alcohol, and water was designated as fraction 3 (F_3). This entire fraction was dried to constant weight

FIG. 10.—Influence of supplementary P fertilization on heavy N fertilization: all cultures received equal heavy doses of N in form of $NaNO_3$; cultures nos. 41 and 104 received equal dosage of extra P; nos. 41 and 44 grown in warm house; nos. 104 and 108 in cool house; P failed to counteract effects of N at higher temperature; chemical analyses made of leaves from this set of cultures.

at 100° C. in an electrically heated oven. Table IV gives the relative proportions of moisture and dry matter in the several samples analyzed.

Table V gives the distribution of the several fractions in the samples analyzed. Particular attention is directed to the fact that the temperature does not seem to have any important effect upon the proportion of lipins (F_1), except where extra phosphorus is present, in which case a high temperature led to an increase in the lipin material. The author regrets not being able to confirm this

FIG. 11.—Influence of supplementary K fertilization on heavy N fertilization: nos. 50 and 113 received equal heavy doses of N in form of $NaNO_3$; nos. 100 and 38 received only "normal" N; all 4 cultures received equal heavy doses of K in form of KCl; nos. 50 and 38 warm house; nos. 100 and 113 cool house; K failed to counteract effects of N at higher temperature.

interesting observation by means of further analyses. The proportion of fraction 2, which might quite properly be designated the metabolic fraction, averages about 10 per cent higher at the higher temperature. The proportion of fraction 3, or storage and skeleton fraction, averages nearly 8 per cent higher at the lower temperature.

F_1 was analyzed for total N and total P. F_2 was analyzed for total N (organic and ammoniacal only), total P, direct reducing

sugars, and for total sugars after mild hydrolysis. Samples 24 and 87 were also analyzed for inorganic phosphorus, using the POWICK-CHAPIN (10) method. F_3 was analyzed for total N, total P, N and P soluble and insoluble in 1 per cent NaOH, phosphoprotein phosphorus, polysaccharides, and cellulose, etc., by

TABLE V

EFFECT OF TEMPERATURE ON DISTRIBUTION OF EXTRACTIVES AND INSOLUBLE MATTER IN BARLEY LEAVES

Culture no. and treatment	Soluble in anhydrous ether (F_1) Percentage	Soluble in hot alcohol and water (F_2) Percentage	Insoluble in ether, alcohol, and water (F_3) Percentage
44. High N warm	8.699	33.017	58.284
24. High N warm	7.885	33.743	58.372
41. High P and N warm	10.433	32.613	56.954
108. High N cool	8.251	30.321	61.428
87. High N cool	8.663	27.823	63.514
104. High P and N cool	7.681	30.279	62.040

difference. The following list gives the methods employed. The details of the several methods are those recommended by KOCH (8) and MATHEWS (9).

Total nitrogen.....Arnold-Gunning method.
Total phosphorus.....Neuman-Pemberton method.
Direct reducing sugars...Bertrand volumetric method (glucose calculated from Munson-Walker tables in MATHEW'S *Physiological Chemistry*).
Total sugars.....Bertrand volumetric method applied to the products of mild hydrolysis with HCl.
Polysaccharides.....Bertrand volumetric method applied to the products of strong hydrolysis with HCl.
Phosphoprotein phosphorus.....Determination of the P precipitable by Mg mixture in an extract made by 48 hours' digestion with 1 per cent NaOH at 37–40° C.

The method for phosphoprotein phosphorus is based upon the discovery by PLIMMER and SCOTT that phosphoproteins can be separated from nucleoproteins through hydrolyzing the former with 1 per cent NaOH, the latter being unattacked by the dilute

alkali. The exact details of the method used on this material are as follows. Weighed samples of F_3 were placed in 300 cc. Erlenmeyer flasks, usually about 0.5 gm., and 1 per cent NaOH, free from phosphorus, was then added at the rate of 100 cc. of NaOH for each 1.0 gm. of substance. The flasks were stoppered and placed in an electric incubator at 37–40° C., where they were allowed

FIG. 12.—Influence of supplementary fertilization with both K and P on heavy N fertilization: no. 120, heavy N+extra K and P, cool house; no. 47, "normal" N+extra K and P, warm house; no. 55, heavy N+extra K and P, warm house; no. 110, "normal" N+extra K and P, cool house; note that "complete fertilizer" failed to counteract effects of heavy N at higher temperature; are not growth effects noted in no. 55 referable to stimulus received at time of germination?

to remain 48 hours. The flasks were shaken about 4 times each day. At the end of the digestion period the insoluble material was filtered off on ashless filter papers and carefully washed with lukewarm water. The combined filtrate and washings were then neutralized to litmus with acetic acid and the PO_4 ions precipitated with magnesia mixture in the presence of an excess of NH_4OH. This precipitation was conducted at a low temperature, the solutions

being allowed to stand in the ice box for 24 hours. At the end of the 24 hour period the magnesium ammonium phosphate was filtered off, washed with 2.5 per cent cold ammonia water, dissolved

FIG. 13.—Influence of variation in fertilization in warm house: N, K, and P indicate that fertilizer dosage is in excess of "normal" A B solution; contrast with results shown in fig. 14, where fertilizer treatment is identical but temperature lower.

FIG. 14.—Influence of variation of fertilization in cool house: contrast with fig. 13

in dilute nitric acid, and the phosphorus then precipitated by means of the molybdate solution. Final determination of the phosphorus was made by means of the Pemberton alkalimetric method.

In order to determine whether or not the material thus extracted by 1 per cent NaOH contained any forms of P not precipitated by magnesia mixture, the phosphorus was determined in the insoluble residue. Similarly total N determinations were made in the insoluble residue from another set of determinations. The difference

FIG. 15.—Influence of extra heavy supplementary P fertilization on heavy N fertilization: no. 41, heavy N and heavy P (warm house); no. 125, heavy N and extra heavy P (cool house); no. 104, heavy N and heavy P (cool house); no. 63, heavy N and extra heavy P (warm house).

between total P (or N) soluble in 1 per cent NaOH gave the P (or N) present in the NaOH extract.

TABLE VI

EFFECT OF TEMPERATURE ON ACCUMULATION OF SOLUBLE CARBOHYDRATES IN BARLEY LEAVES (RESULTS OF ANALYSIS OF F_2)

CULTURE NO., TREATMENT, AND TIME OF SAMPLING	DIRECT REDUCING SUGARS (AS GLUCOSE)		TOTAL SUGARS AFTER MILD HYDROLYSIS (AS GLUCOSE)		PERCENTAGE CONCENTRATION OF TOTAL SUGARS (AS GLUCOSE) IN TOTAL WATER IN TISSUE
	Percentage F_2	Percentage total leaf	Percentage F_2	Percentage total leaf	
44. High N 4 P.M., April 24, 1918 warm.....	15.99	5.27	29.41	9.71	0.15252
24. High N 9 A.M., April 25, 1918 warm.....	19.83	6.69	26.84	9.05	0.15342
41. High P and N 3 P.M., April 25, 1918 warm..	10.62	3.46	18.55	5.05	0.06535
108. High N 6 P.M., April 24, 1918 cool.....	20.20	6.11	45.79	13.88	0.26007
87. High N 10 A.M., April 24, 1918 cool.....	23.17	6.44	36.99	10.29	0.18985
104. High P and N 5 P.M., April 24, 1918 cool...	25.08	7.59	39.90	12.08	0.22142

Tables VI–XII contain the results of the different determinations. Table XIII gives the proportion of skeletal material in

F_3, calculated as the difference between the total amount of the fraction, and the sum of the protein and starch in the fraction.

TABLE VII

EFFECT OF TEMPERATURE ON ACCUMULATION OF POLY-SACCHARIDES IN BARLEY LEAVES

Culture no. and treatment	Percentage of F_3	Percentage of total leaf
44. High N warm	21.16	12.33
24. High N warm	21.64	12.63
41. High P and N warm	20.43	11.63
108. High N cool	25.51	15.67
87. High N cool	23.60	14.99
104. High P and N cool	24.14	14.97

TABLE VIII

EFFECT OF TEMPERATURE ON AMOUNT OF NITROGEN, PHOSPHORUS, PROTEIN, AND PHOSPHOPROTEIN PHOSPHORUS IN BARLEY LEAVES (PERCENTAGE OF TOTAL DRY WEIGHT)

Culture no. and treatment	Total N	Total P	Total protein (Percentage N in $F_3 \times 6.25$)	Phospho-protein phosphorus
44. High N warm	2.9779	0.6944	12.965	0.1167
24. High N warm	2.7007	0.6789	11.277	0.1411
41. High P and N warm	3.4661	0.7988	14.425	0.0795
108. High N cool	2.5610	0.5479	12.865	0.0543
87. High N cool	2.6065	0.5711	13.159	0.0832
104. High P and N cool	2.5945	0.6532	12.008	0.0996

TABLE IX

EFFECT OF TEMPERATURE ON AMOUNT AND DISTRIBUTION OF NITROGEN IN BARLEY LEAVES

Culture no. and treatment	F_1 soluble in anhydrous ether			F_2 soluble in hot alcohol and water			F_3 insoluble in alcohol, ether, or water		
	Percentage fraction	Percentage total leaf	Percentage total N in leaf	Percentage fraction	Percentage total leaf	Percentage total N in leaf	Percentage fraction	Percentage total leaf	Percentage total N in leaf
44. High N warm	1.4993	0.1304	4.38	2.3420	0.7732	25.96	3.559	2.0743	69.66
24. High N warm	1.3081	0.1021	3.78	2.1312	0.7191	26.62	3.237	1.8795	69.60
41. High P and N warm	2.6700	0.2785	8.03	2.6970	0.8795	25.377	4.053	2.3081	66.60
108. High N cool	1.4142	0.1167	4.55	1.2730	0.3859	15.07	3.351	2.0584	80.38
87. High N cool	1.2520	0.1084	4.16	1.4110	0.3925	15.06	3.315	2.1054	80.78
104. High P and N cool	1.3205	0.1014	3.91	1.4130	0.4278	16.49	3.329	2.0653	79.60

TABLE X

EFFECT OF TEMPERATURE ON AMOUNT AND DISTRIBUTION OF PHOSPHORUS IN BARLEY LEAVES

CULTURE NO. AND TREATMENT	F_1 SOLUBLE IN ANHYDROUS ETHER			F_2 SOLUBLE IN HOT ALCOHOL AND WATER			F_3 INSOLUBLE IN ALCOHOL, ETHER, AND WATER		
	Percentage fraction	Percentage total leaf	Percentage P in leaf	Percentage fraction	Percentage total leaf	Percentage total P in leaf	Percentage fraction	Percentage total leaf	Percentage total P in leaf
44. High N warm	0.7369	0.0641	9.23	0.8683	0.2866	41.28	0.5898	0.3437	49.49
24. High N warm	0.6847	0.0539	7.93	0.8211	0.2770	40.80	0.5960	0.3478	51.27
41. High P and N warm	0.7857	0.0819	10.26	1.3394	0.4368	54.69	0.4918	0.2800	35.05
108. High N cool	0.7739	0.0638	11.65	0.5352	0.1622	29.62	0.5239	0.3218	58.73
87. High N cool	0.7247	0.0627	10.99	0.5096	0.1417	24.80	0.5614	0.3565	64.21
104. High P and N cool	0.7975	0.0612	9.37	0.9602	0.2907	44.50	0.4856	0.3012	46.13

TABLE XI

EFFECT OF TEMPERATURE UPON SOLUBILITY OF F_3 NITROGEN OF BARLEY LEAVES IN 1 PER CENT NaOH (MATERIAL DIGESTED WITH 1 PER CENT NaOH FOR 48 HOURS AT 37–40° C.)

CULTURE NO. AND TREATMENT	SOLUBLE NITROGEN			INSOLUBLE NITROGEN		
	Percentage fraction	Percentage total leaf	Percentage total N in leaf	Percentage fraction	Percentage total leaf	Percentage total N in leaf
44. High N warm	0.910	0.5303	17.81	2.649	1.5440	51.85
24. High N warm	0.8570	0.4902	18.17	2.380	1.3892	51.43
41. High P and N warm	1.126	0.6411	18.50	2.927	1.6670	48.10
108. High N cool	0.873	0.5361	20.93	2.478	1.5221	59.43
87. High N cool	1.052	0.6681	26.03	2.263	1.4373	54.75
104. High P and N cool	0.923	0.5726	22.07	2.406	1.4926	57.53

TABLE XII

EFFECT OF TEMPERATURE UPON SOLUBILITY OF F_3 PHOSPHORUS OF BARLEY LEAVES IN 1 PER CENT NaOH (MATERIAL DIGESTED WITH 1 PER CENT NaOH FOR 48 HOURS AT 37–40° C.)

CULTURE NO. AND TREATMENT	SOLUBLE PHOSPHORUS (BY DIFFERENCE)			INSOLUBLE PHOSPHORUS		
	Percentage fraction	Percentage total leaf	Percentage total P in leaf	Percentage fraction	Percentage total leaf	Percentage total P in leaf
44. High N warm	0.2292	0.1335	19.22	0.3606	0.2102	30.27
24. High N warm	0.1994	0.1163	17.13	0.3966	0.2315	34.10
41. High N and P warm	0.1959	0.1115	13.96	0.2959*	0.1685*	21.09
108. High N cool	0.1132	0.0696	12.70	0.4107	0.2522	46.03
87. High N cool	0.1807	0.1148	20.10	0.3807	0.2417	42.32
104. High N and P cool	0.1666	0.1039	15.91	0.3190†	0.1979†	30.30

* Poor duplicates. † One analysis only, duplicate lost.

TABLE XIII

EFFECT OF TEMPERATURE UPON AMOUNT OF CELL WALL MATERIAL, ETC. $F_3-[(N \text{ IN } F_3 \times 6.25)+(\text{STARCH IN } F_3)]$; EXPRESSED AS PERCENTAGE OF TOTAL DRY WEIGHT OF LEAF

Culture no. and treatment	Cell wall material, etc.	Ratio of supporting tissue (cell walls, etc.) to all other plant substances, including water
44. High N warm	32.99	0.0470
24. High N warm	34.47	0.0525
41. High P and N warm	30.90	0.0367
Average warm	32.78	0.0454
108. High N cool	32.89	0.0539
87. High N cool	35.36	0.0581
104. High P and N cool	34.16	0.0558
Average cool	34.13	0.0559

TABLE XIV

EFFECT OF TEMPERATURE ON DISTRIBUTION OF PHOSPHORUS; SUMMARY TABLE

Material	No. 24, high N, warm		No. 87, high N, cool	
	Percentage total leaf	Percentage total P	Percentage total leaf	Percentage total P
Lipoid P, F_1	0.0539	7.94	0.0627	10.99
Phosphate P, F_2	0.2105	31.01	0.0714	12.80
Organic P, F_2	0.0665	9.80	0.0703	12.31
Phosphoprotein P, F_3	0.1411	20.80	0.0832	18.38
Nucleoprotein P, F_3	0.2067	30.45	0.2833	49.62
Total P	0.6787		0.5709	

Results of chemical analysis

LIPIN FRACTION (F_1).—The results given in table V indicate that the temperature has very little effect upon the amount of lipins, except in the case of a high phosphorus supply, where the percentage of lipins is decidedly higher. This fact is possibly correlated with the higher percentage of phospho-lipin phosphorus in the entire leaf, as shown in the third column of table X, and the higher percentage of lipin N as shown in the third column of table IX. Since the proportion of lipin P is practically the same for both temperatures in the case of the high nitrogen series, these data lead to the conclusion that the lipin fraction is not an important growth determinant. The writer recognizes the desirability of more data.

Alcohol-water soluble fraction (F_2).—Table V shows a distinctly higher average percentage of these extractives at the higher temperature, although the order of difference is not large. When, however, the composition of this fraction is examined certain striking differences are noted. The high temperature leaves contain a much lower percentage of both total and reducing sugars (table VI) and a lower percentage of polysaccharides (table VII). The high temperature leaves contain about twice as much nitrogen (as determined by the unmodified Arnold-Gunning process) as do the low temperature leaves (table IX). In other words, the amount of active metabolic nitrogen, such as amino acids, polypeptides, and simpler water soluble proteins, is much higher at the higher temperature. The amount of nitric N is also higher at the higher temperature, as was indicated when the modified Arnold-Gunning process was used. The results of the nitric N determinations are not reported in this paper. The high temperature leaves also contain nearly twice the percentage of alcohol-water soluble phosphorus. Duplicate determinations on one set of samples (nos. 24 and 87) indicated that this difference was very largely due to the much higher percentage of inorganic phosphorus at the higher temperature. These results are appended, although it is recognized that more data are needed before any sweeping generalizations can be made. The Powick-Chapin method was used in this determination.

Fig. 16.—Influence of temperature on maturation (photographed May 16): no. 12, "normal" fertilization (warm house); no. 74, "normal" fertilization (cool house).

	Total P		Inorganic P	
	Percentage of fraction	Percentage of entire leaf	Percentage of fraction	Percentage of entire leaf
No. 24	0.8211	0.2770	0.6240	0.2105
No. 87	0.5096	0.1417	0.2567	0.0714

Fraction 3.—The higher amount of polysaccharides at the lower temperatures has been noted. Table V shows that the leaves grown at the lower temperature contain a distinctly higher average percentage of this fraction, although the order of difference is not large. Tables IX and X show that there is no important

Fig. 17

Fig. 18

Figs. 17–18.—Fig. 17, influence of heavy N and heavy K on maturation (photographed May 16): no. 49, heavy N+heavy K (warm house); no. 112, heavy N+heavy K (cool house); comparison with other sets not shown indicate that K has no effect in causing difference; contrast heavy N cultures with normal N cultures of fig. 16; fig. 18, influence of heavy N and extra heavy P on maturation (photographed May 16): no. 63, heavy N+extra heavy P (warm house); no. 126, heavy N+extra heavy P (cool house); contrast with nos. 63 and 125 (same treatment) in fig. 15.

difference in the percentage of either N or P at the different temperatures. The amount of phosphoprotein phosphorus seems to run somewhat lower at the lower temperature (table VIII).

In five out of six cases (cf. column 3, table XII, with column 5, table VII) the amount of phosphorus in the NaOH extract exceeded the phosphorus precipitable from that extract by 1 per cent NaOH, indicating that either some organic phosphorus compounds had

been dissolved by the NaOH but had not been hydrolyzed, or that the magnesia mixture failed to give quantitative precipitations of the PO_4 ions under the conditions of the experiment.

Table IX reports a study of the solubility of the F_3 nitrogen in 1 percentage NaOH. The results are inconclusive, but are reported for the sake of completeness.

The calculations reported in table XIII are self-explanatory. It will be noted that the average proportions of framework material are considerably higher at the lower temperature. Microchemical examination of median cross-sections of the leaves and of the culms showed a greater degree of lignification of the xylem bundles at the lower temperature, a fact of added significance. Lignification of the vessels in the culm adds greatly to the strength of the stem. Referring to the enormous differences in growth habit as shown in the figures, we may conclude that the upright habit at the lower temperature is due to: (1) a greater proportion of culm to leaf; (2) a greater proportion of skeletal material in the leaf; (3) a greater degree of lignification of conductive tissues in both leaf and culm. These obvious anatomical facts, however, are but the expression of a difference in metabolic equilibria, especially the nitrogen-carbohydrate ratio.

Discussion

The experiments reported in this paper, as well as the results of earlier investigators, reopen the question as to just what is meant by an optimum germination temperature. The classical investigations of HABERLANDT on germination temperature place the optimum at the temperature which most quickly permits the emergence of the radicle and plumule; in fact, practically all germination studies have been based upon this as the optimum. These optimum temperatures, at least for the cereals, are evidently too high to insure a future normal development. The writer believes that the course of development is to a large extent predetermined at a very early stage in the development of the plant by the chemical equilibria within the seedling, especially the nitrogen-carbohydrate ratio. These equilibria within the plant, like chemical reactions *in vitro*, are conditioned by the temperature and concentrations of the reacting substances. It seems likely that a high temperature

and a high nitrogen supply at an early stage in the development of the barley plant so shifts the equilibrium toward excessive vegetation as to prevent the normal tendency toward reproduction. Some other factor must be altered, therefore, as, for example, the water supply, if such plants are to be thrown into reproduction.

An investigation of the nitrogen-carbohydrate ratio at a different stage in the development of seeds and seedlings furnished with varying concentrations of nitrogenous compounds will probably throw considerable light upon these questions.

Conclusions

1. The excessive leaf production in the high temperature barley is caused by the high concentration of nitrates in the nutrient supplied.

2. Nitrate nitrogen in the nutrient begins to affect the subsequent course of development at high temperatures at the time of germination, or at least at a very early stage in the development of the plant. The tendency to excessive vegetation thus inaugurated cannot be counteracted by the addition of phosphorus or potassium salts.

3. The effect of the nutrient supply is reflected in the composition of the active organ, the leaf. The following equations represent the main facts revealed by chemical analysis of the leaf:

High heat supply + high nitrogen supply in nutrient solution = high soluble nitrogen in leaf + low soluble carbohydrate = excessive vegetation and little culm formation.

Low heat supply + high nitrogen supply in nutrient solution = low soluble nitrogen in leaf + high soluble carbohydrate = normal vegetation and normal culm formation.

The writer gratefully acknowledges his indebtedness to Professor William Crocker for helpful advice and criticisms; to Professor F. C. Koch for valuable advice and laboratory facilities; and to the Department of Zoölogy of the University of Chicago for facilities afforded in their greenhouses.

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N.D.

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PHYSIOLOGICAL STUDY OF MAPLE SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 260

(WITH TWO FIGURES)

H. A. JONES

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Introduction

The appearance of two taxonomic species within the same genus is not always a criterion of similar physiological or ecological behavior. The seeds of two closely related species, as those of the sugar and river maples (*Acer saccharum* Marsh. and *A. saccharinum* L.), show a striking contrast in season of maturity, reaction to external conditions, chemical composition, and in their physiological behavior in general. The sugar maple matures its seeds in the fall, and these must pass through a well defined period of after-ripening before germination can take place. The storage substances are mainly protein and fat, with a small amount of carbohydrate present. On the other hand, the river maple ripens its seeds in the spring. The seeds germinate almost immediately upon a moist substratum, but if allowed to desiccate for some time under ordinary atmospheric conditions they soon lose their power of germination. A very small percentage of fat and protein is present, starch being the chief storage product.

It is a matter of common observation that many mature seeds and spores soon lose their power to germinate when subjected for varying periods to atmospheric desiccation. In a great many tropical seeds death follows atmospheric drying. In our own region the seeds of the willow and cottonwood are usually cited as the classic examples of death due to desiccation shortly after seed fall. The cottonwood gives low percentage of germination and low seedling vigor after two weeks of desiccation in laboratory air, while after three weeks seeds fail to germinate when placed in the most favorable germinative conditions. Cottonwood seeds, however, are in a high state of metabolic activity when first shed.

At 30° C. on moist filter paper the fresh seeds will usually give 100 per cent germination within 24 hours. The hypocotyls will attain a length of 8–9 mm., and the cotyledons will be entirely spread. SCHRÖDER (23) states that seeds of *Caltha palustris* failed to germinate after 11 weeks of storage over sulphuric acid and after 20 weeks of storage in the ordinary atmosphere. DELAVAN (8), working with the oaks and hickories, concludes that a cold even temperature, although the atmosphere be moist, is better than warm dry storage of seed. Seeds of *Oxalis*, elm, river maple, hornbeam, birch, beech, chestnut, and probably many others have their germinative power lowered or lost entirely by varying periods of desiccation.

Heretofore no work has been done on seeds, sensitive to drying, regarding the exact or approximate water content at the time of death. Furthermore, it has never been demonstrated whether loss of viability is due in part to temperature or entirely to desiccation effects.

Investigation

RIVER MAPLE (*Acer saccharinum* L.)

In the Chicago region *Acer saccharinum* matures its seeds the latter part of May or early in June, varying with the season. At the time of fall the seeds contain approximately 58 per cent of water, being almost fully imbibed. The seeds soon germinate if they lodge upon a moist substratum, but if they are subjected to desiccation there is an immediate reduction of the moisture content, and their viability is lost long before an air-dry condition is attained. The seeds of the river maple were chosen for this study because they are large, making it possible to obtain material readily in sufficient quantities for chemical analysis. The period of time between maturing and loss of viability is of moderate duration, permitting a study of internal changes accompanying desiccation; also seeds are abundant and easily collected. In all cases where reference is made to the maple fruit the seed plus the ovary wall is taken into consideration. Seed refers to the embryo plus the integuments. In all storage conditions the entire maple fruit was used; this holds for both the river and sugar maple. The criterion

for the beginning of germination is the protrusion of the tip of the hypocotyl through the integuments.

Water and temperature relations

Fruits were collected at time of shedding and stored at various constant temperatures from 0 to 40° C. At 25° C. and above fruits were stored in open wire baskets. At 20° C. and below they were stored in loosely covered cans which contained a considerable quantity of calcium oxide. The lime facilitated drying at the lower temperatures, besides preventing the accumulation of an excess carbon dioxide pressure about the seeds. By August 26, 1918, all seeds desiccated at 0–40° C. had lost their

TABLE I

LIFE DURATION OF SEEDS STORED AT VARIOUS DRYING TEMPERATURES

Storage temperature	Life duration*
35° C.	6 days
30	8
25	22
20	20
10	49
0	92

* At 25° C. the humidity of the air was considerably higher, and drying somewhat slower than at 20° C., accounting for increased life duration.

ability to germinate. In all cases seeds were considered to have lost their viability when 80 per cent failed to germinate when placed on moist filter paper at 30° C., all seeds having either germinated or decayed. From 0 to 35° C. the seeds lost their viability when the water content was reduced to 30–34 per cent. So far as could be determined, the various temperatures from 0 to 35° C. for desiccation do not appear to raise or lower the critical point of water content. At 40° C. death does not seem to be due to desiccation. Seeds turn black in a short time, killing apparently being due to the destructive action of this high temperature. One apparent effect of increasing temperatures (0–35° C.) is the shortening of the desiccation period, no change being evident in

the percentage of water at several temperatures at the time of loss of viability.

Seeds have a high metabolic activity at time of fall. Where viability and vigor are so closely allied with high water content, it is logical to suppose that the initial vigor can be retained for some time by holding the water percentage at the initial content, and by lowering the metabolic activity. Seeds at maturity and for some time thereafter give off considerable amounts of CO_2. For a number of samples at time of fall the yield of CO_2 was estimated as approximately 7 mg. per gram of dry weight per 24 hours at 25° C. If we consider 7 mg. as the amount of CO_2 respired in 24 hours at 25° C., the seeds would soon exhaust their store of food if the initial activity were maintained. The carbohydrate present would be entirely exhausted and the seeds die of starvation within approximately 120 days if this initial intense respiratory activity were maintained. At this rate it would be impossible to hold seeds just below the point of saturation at the higher temperature for any great length of time. Seeds, however, can be held for some time stored over water at low temperatures. Seeds harvested in the spring of 1917 were stored over water in desiccators at 10° C., and continued to give 95–100 per cent germination until November 1917. There was, however, an abnormal development of the hypocotyl during the latter part of the storage period at 10° C. No alkali was placed in the desiccators to prevent CO_2 accumulation, so it is impossible to say just what part was played by the carbon dioxide in the preservation of the seeds at this temperature. In the spring of 1918 seeds were stored over water in a large desiccator at 0° C. A bottle of strong alkali was also placed in the desiccator to prevent accumulation of a CO_2 blanket. These seeds were discarded after 102 days' storage, and at this time seeds were giving 100 per cent germination. They had retained their initial vigor and appeared to be normal in every respect. Perhaps many other seeds of this general behavior would retain their viability and vigor for considerable periods when placed in similar storage conditions. Seeds can be kept for a considerable period at temperatures just below the freezing point. After 50 days seeds stored at −5° C. gave good germination. At this low temperature care

must be taken that water does not come into contact with the outer walls of the fruit or integuments, as ice formed on the latter appears to inoculate the subcooled tissue below, and freezing to death results.

Respiration

Respiration was determined on newly collected seeds, on seeds desiccated at 25° C., and on germinating seeds. Determinations were made on the desiccating seeds every second day until viability was lost, and for several weeks thereafter. All respiration experiments were conducted at 25° C., as this temperature was thought to correspond very closely with the average temperature to which the seeds would be subjected under natural conditions. The method of determining the carbon dioxide given off was that described by GRAFE (12), with slight modifications. In general the method consists in pulling carbon dioxide free air over the respiring material through a column of barium hydroxide. The barium hydroxide solution is held by a Reiset tube. The air is drawn through slowly and uniformly. This is accomplished best by the air replacing water which is slowly siphoned out of a large demijohn by means of a capillary tube. At the end of a determination the barium carbonate was allowed to settle and an aliquot part (25 cc.) of the 100 cc. of barium hydroxide was pipetted off and titrated with N/20 oxalic acid. Phenolphthalein was the indicator used.

If the intensity of respiration may be used as a criterion of metabolic activity, then the seeds of the river maple at time of fall are in high state of metabolism. In the desiccating seeds there is a fall the first few days in respiratory activity, and then a gradual rise until a maximum is reached. This maximum is retained for several days, then there is a gradual decline, until only a trace of carbon dioxide is given off. This secondary rise in respiratory intensity may accompany increased starch hydrolysis. It will be seen later that accompanying desiccation there is a great increase in sucrose, due to starch hydrolysis. The later fall in respiratory activity is probably caused by a deficiency of water. The greatest respiratory activity was obtained on the desiccating seeds with a water content of approximately 44 per cent. There is no marked

degeneration of the respiratory enzymes during this fall, because when dead seeds are placed in germinative conditions the respiration again mounts to a high value, giving off 8.84 mg. of carbon dioxide per gram of dry weight in 24 hours. It is not known, however, just what percentage of the carbon dioxide given off in the latter case was due to bacterial action. HAAS (13) found that the marine alga *Laminaria*, in the presence of certain reagents, respired more rapidly after death than in the living condition. MAIGE and NICOLAS (17) have done considerable work on respiration in correlation with the state of turgidity of certain plant organs,

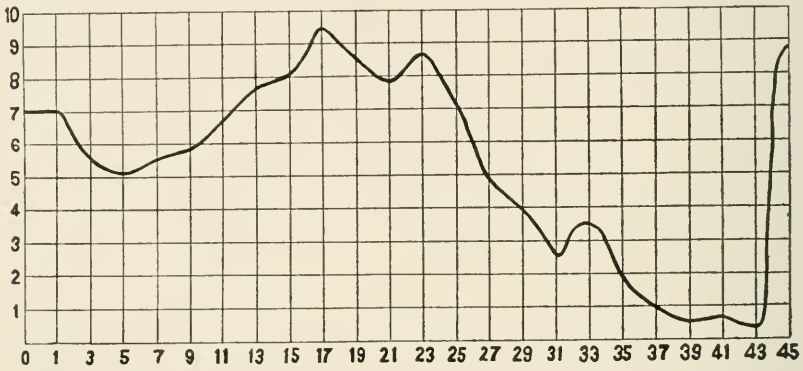

FIG. 1.—Respiration curve for seeds desiccating at 25° C.; mg. of CO_2 given off in 24 hours per gm. dry weight plotted on ordinates; time of desiccation in days plotted on abscissae; great rise in respiration after forty-third day due to placing desiccated seeds (dead at time) under favorable germinative conditions.

as buds, leaves, and embryos. They find in material taken directly from the tree increased carbon dioxide production with increased turgescence, also for decreased turgescence, and usually an increase in respiration when decrease was followed by an increase. Fig. 1 represents the trend of respiration during 43 days of desiccation. The sudden rise on the forty-fifth day shows respiratory activity of seeds after being placed in germinative conditions.

To determine the respiratory activity of germinating seeds, newly collected seeds were planted in the dark at 25° C. The respirometer used was a 500 cc. graduated cylinder. This was half filled with shredded filter paper, previously well sterilized. The

filter paper was packed very loosely in the graduated cylinder. The seeds were washed with distilled water and planted near the surface of the paper, about midway between the top and bottom of the chamber. A small amount of water was run into the respirometer. The top was stoppered and supplied with an inlet tube which extended to the bottom of the chamber and brought in the carbon dioxide free air, and with an exit tube which carried the carbon dioxide laden air to the Reiset tube. The seedlings were grown in the dark and consequently there was no food manufactured. Storage food only was used up in respiration.

The respiratory activity of the germinating seeds reaches a maximum about the eighth day at this temperature. At this time the seedling has elongated considerably, the radicle having attained a length of 7–10 cm., varying considerably with the individual. After the eighth day respiration decreases gradually. Seeds stored for several weeks at a low temperature (0° C.) and then transferred to a high temperature (25° C.) in germinative conditions show a very high initial respiratory intensity, which soon drops to normal, and then again increases. PALLADIN (20) found that transferring the tips of etiolated bean seedlings from a lower to a higher and also from a higher to a lower temperature increased the respiratory activity. According to APPLEMAN (1), tubers stored at low temperature for several weeks and then transferred to room temperature respire more intensely than tubers of the same lot not subjected to the cold storage conditions. He thinks this increased respiration might result from the increased accumulation of sugar at the lower temperatures.

Fig. 2 shows the march of respiration during the first 14 days of germination in the dark. In general this curve agrees with that found by RISCHAWI (21) for the respiration of the wheat seedling growing in the dark, but is quite different from that found for the bean.

Catalase activity

The apparatus used for catalase determinations was a modified form of the one used by APPLEMAN (2). Determinations were made upon fresh seeds, seeds desiccating at 25° C., and also seeds germinating in the dark at 25° C. Entire seeds were used in all cases.

Material was weighed, then ground in a mortar with a small amount of quartz sand and a knife point of calcium carbonate for exactly 2 minutes. This emulsion was then washed with the aid of 10 cc. of distilled water into a 200 cc. wide-mouthed bottle. The latter was then corked and plunged into a water bath kept at 25° C. The commercial form of Oakland dioxygen was used at all times. This dioxygen gives an acid reaction. To neutralize the acidity a small excess of calcium carbonate is added to the dioxygen just

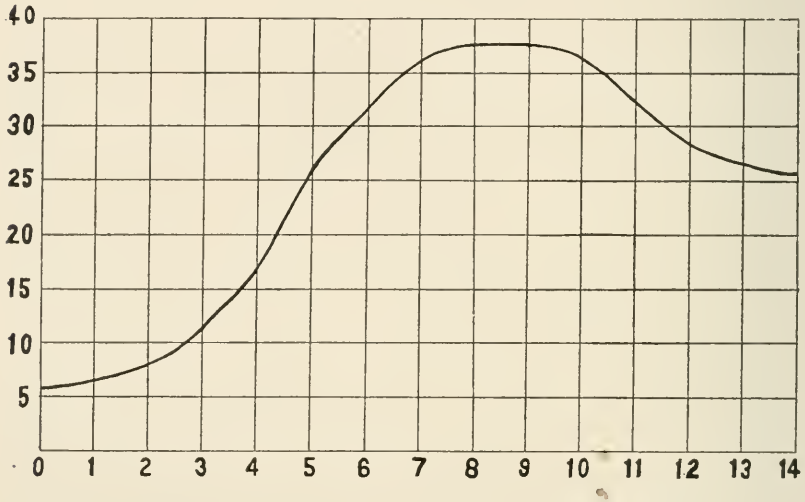

FIG. 2.—Respiratory curve for first 14 days of germination in dark at 25° C.; time of germination in days plotted on abscissae and mg. of CO_2 given off in 24 hours per gm. of dry weight plotted on ordinates.

before using. If the acidity is not corrected, the catalase activity is reduced approximately one-half. A small separatory funnel inserted in the cork of the bottle holds the dioxygen. The latter is run into the ground tissue when the dioxygen and pulp have reached the same temperature as the water bath. The material is then shaken uniformly for 10 minutes by means of a small motor. The oxygen liberated is collected over water at atmospheric pressure in a 100 cc. burette. Table II shows the catalase activity at various times during desiccation and the early stages of germination.

Catalase activity increases slightly during the first few days of desiccation, but decreases gradually thereafter. This activity seems to align itself in a general way with respiratory activity, which remained high for a considerable time. With germination the catalase activity increases enormously, appearing to be closely correlated with metabolic activity. There is not a sudden drop in the catalase activity at the time of loss of viability, as one might

TABLE II

CATALASE ACTIVITY ACCOMPANYING DESICCATION AND FIRST STAGES OF GERMINATION

CONDITION OF SEEDS OF SEEDLINGS	NO. OF CC. OF O_2 GIVEN OFF BY 1 GM. OF DRY WEIGHT IN	
	5 minutes	10 minutes
Fresh seeds collected May 25 (1918)	952	1248
Desiccated at 25° C. for 3 days.....	1035	1373
" " " " 5 "	1106	1447
" " " " 10 "	977	1341
" " " " 14 "	1075	1359
" " " " 18 "	1022	1259
" " " " 22 "	868	1098
" " " " 26 "	731	979
" " " " 34 "	688	909
" " " " 42 "	461	593
Desiccated in laboratory for 8 months..	380	500
Seedlings with radicle 1 cm. long.....	1245	1565
" " " 2 " "	1717	2055
" " " 3 " "	2106	2566
" " " 4 " "	2438	3060
" " " 5 " "	3216	4472

expect, but a gradual decrease correlated with respiratory activity and water loss. After a storage for 8 months under laboratory conditions the catalase activity was reduced more than one-half below that of the fresh seed.

Oxidase and peroxidase

Peroxidase activity is very intense in the fresh seeds. A dark blue color is obtained immediately upon addition of alcoholic solution of benzidine and a drop of dioxygen. As desiccation progresses there is a gradual decrease in peroxidase activity. In one-year-old dead seeds there is only a very pale blue color evident

about the vascular tissue when this method is used. No oxidase could be detected by the ordinary qualitative chromogenic methods in either the living or desiccated seeds.

Chemical analysis

In the following analysis seeds were collected from the same tree in order to eliminate differences due to individual variation. The collection was made in the spring of 1917. Fresh seeds were immediately placed in 95 per cent redistilled alcohol, enough being added to make the final volume of alcohol 80 per cent. One-half gram of calcium carbonate was added to guard against possible acid hydrolysis. In the final calculation the calcium carbonate was considered as being in the insoluble fraction. In general the method of extraction and analysis is that outlined by KOCH (16), but a few modifications were found necessary.

TABLE III

Fraction	Fresh seeds	Desiccated seeds
Percentage F_3 of total dry weight ..	79.05	65.56
" F_2 " " " " ..	15.8	30.31
" F_1 " " " " ..	5.15	4.13

The tissue was ground, and then extracted with hot 95 per cent alcohol for four hours, followed by 1-hour ether extraction. The alcohol-ether insoluble material was then heated in water for one hour on the steam bath. The water was evaporated down, alcohol again added, and returned to extraction cups for a 24-hour alcohol extraction and 1-hour ether extraction. The alcohol and ether extracts were combined, evaporated to dryness, and then extracted with anhydrous ether. This ether extract is known as F_1; the residue from the ether extract is F_2; the alcohol-ether insoluble material is F_3. F_3 was dried in the oven at 103° C. for 5 days, then cooled and weighed.

The 1917 seeds were desiccated in the laboratory. No attempt was made to maintain a constant temperature. The seeds failed to germinate after 18 days, when the water content had dropped to approximately 34 per cent. The desiccated seeds were treated in the same manner as the fresh seeds. Table III shows the

percentage variation in the various fractions accompanying desiccation.

It can readily be seen that accompanying desiccation under laboratory conditions there is a great increase in F_2. One would be led to expect quite the contrary, as condensation is quite commonly associated with desiccation in plants. Table IV shows more in detail to what this increase is due.

During the period of desiccation there has been an enormous increase in the percentage of sucrose. Accompanying this increase

TABLE IV

ANALYSIS OF FRESH AND DESICCATED SEEDS

MATERIAL	PERCENTAGE TOTAL DRY WEIGHT	
	Fresh seeds	Desiccated seeds
Free reducing sugar	0.53	0.43
Sucrose (calculated as invert sugar)	4.53	14.41
Starch	48.18	35.42
F_1 Nitrogen	0.03	0.02
F_2 Nitrogen	0.65	0.80
F_3 Nitrogen	3.36	3.28
F_1 Phosphorus	0.03	0.02
F_2 Phosphorus	0.18	0.31
F_3 Phosphorus	0.50	0.35

is a corresponding decrease in the starch content. Free reducing sugars remain approximately the same. In the desiccated seeds we also find a slight increase in phosphorus and nitrogen in F_2. The nitrogen here represents merely the Kjeldahl nitrogen.

SUGAR MAPLE (*Acer saccharum* Marsh.)

Historical

A very different type of behavior is found when the seeds of the sugar maple are considered. Germination here is initiated by a distinct period of after-ripening. Investigators generally have used the term "after-ripening" as referring to the series of chemical or physical changes occurring within the embryo or associated structures, which bring to a close the dormant period and make germination possible. The factors operating to cause delayed germination in most types of seed dormancy studied to the present

time have been treated in some detail by CROCKER (5). Seeds that have dormant periods fall naturally into two groups: (1) seeds, like certain members of the Leguminosae, have embryos capable of immediate germination, but dormancy is here induced by associated structures like the seed coats or pericarp; (2) the embryo itself may be the cause of delayed germination. The second type of dormancy may be due either to an immature embryo, as found in *Ceratozamia* (4) and *Ilex opaca* (14), the former often being shed at the time of or shortly after fertilization, while in the holly the embryo is merely a globular undifferentiated group of cells at the time of seed fall; or dormancy may appear in apparently fully matured embryos, as is the case in some members of the Rosaceae. The seeds of the sugar maple fall into the latter group, having a dormant, morphologically mature embryo.

DAVIS and ROSE (7) found that in nature *Crataegus mollis* has a dormant period of a year or more. This period of dormancy can be shortened considerably by removing the carpel and testa. It is doubtful whether any such interrelation exists between the embryo of the sugar maple and its inclosing structures.

The sugar maple sheds its fruit in the fall, after the first few hard frosts. When given the most favorable conditions for germination at time of fall the seeds fail to respond. The seeds must be kept at a low temperature, with plenty of moisture present for a considerable period of time for after-ripening to reach completion. Under natural conditions, if the seeds are kept moist during the fall and winter, after-ripening will be complete the latter part of February or early part of March.

Investigation

The object of the investigation was twofold: (1) to determine the optimum temperature and water relations for after-ripening; and (2) to determine the changes taking place within the embryo during the after-ripening period. The fruit of the sugar maple was collected the latter part of September and early part of October direct from the trees in the Chicago region and northern Indiana. Fruits were stored dry in wire baskets at various temperatures from -5 to $+30°$ C.; others were stored in desiccators over water at

5° C. and 10° C.; also, some were stored out of doors on the surface of the ground and kept covered during the fall and winter to prevent drying.

Temperature and water relations

When seeds were stored dry, in no case, regardless of storage temperature, did after-ripening reach completion; that is, no dry stored seeds would germinate when placed in Petri dishes on moist cotton at favorable germination temperatures. All dry stored seeds required a prolonged stay at low temperatures with plenty of moisture present to completely after-ripen. DAVIS and ROSE found that after-ripening in the haw proceeded best at temperatures near 5° C. The sugar maple was also found to after-ripen best at about this temperature.

In January, after three and a half months of dry storage, specimens were removed from each of the dry stored samples, and placed at 5° C. under good germinative conditions. The pericarp was removed and the seeds that had been dry stored at 5° C. were the first to complete their period of after-ripening, most of the seeds completing after-ripening during the fifth week. The seeds, however, do not after-ripen uniformly; some precede and others follow the general average time. Seeds dry stored at −5° C. take the longest time to complete their period of after-ripening, taking 4–5 weeks longer than seeds dry stored at 5° C. Seeds dry stored at 10–30° C. after-ripen more slowly than seeds stored at 5° C., and more quickly than seeds stored at −5° C. In other words, seeds dry stored at 5° C. have progressed farthest, and those stored at −5° C. have progressed least in the process of after-ripening at their respective storage temperatures. The factor limiting the complete after-ripening in the dry stored seeds at low favorable temperatures is a deficient water supply. Only in the presence of sufficient water can the various processes go progressively on to complete after-ripening.

Fruits stored on the surface of the ground were subjected to the temperature ranges of the soil surface. The seeds, however, were kept saturated, due to the extremely wet fall and winter. At time of fall seeds had a water content of 55 per cent, and during the entire fall and winter the water content remained at 55–57 per

cent. In the seeds stored out of doors and in desiccators over water there was no indication of increased water holding capacity accompanying after-ripening. Seeds stored in desiccators at low temperatures over water are completely after-ripened several weeks before seeds stored out of doors. Table V shows how after-ripening progressed in seeds stored out of doors. As after-ripening progressed, less and less time was required for the completion of this process when placed in the germinator at 10° C.

TABLE V

Put to germinate at 10° C.	Percentage of germination after number of days indicated											
	1	2	3	4	5	6	8	12	17	26	30	35
January 16, 1918	....	....	....	....	....	....	....	....	....	....	68	88
February 4	....	....	....	....	....	....	....	39	83	92	....	....
February 28	19	50	....	....	....	....	92	....	....	....	....	....
March 5	40	67	77	85	95	97	100	....	....	....	....	....

Seeds after-ripened out of doors and at 5° C. are more vigorous than seeds after-ripened at slightly higher temperatures (10° C.). Dry stored seeds at low temperatures are more vigorous when after-ripened than seeds previously dry stored at high temperatures. This question of vigor should be given more attention than it has been given up to the present time. There is something very significant in the fact that maximum vigor can be obtained by after-ripening seeds at a temperature so much below the optimum germination temperature and at a temperature which we consider retarding to metabolic activity in general. Poor germination and high seedling mortality can be replaced by good germination and vigorous seedlings when the most favorable temperature (about 5° C.) and water relations are used for after-ripening. After-ripening and germination is a continuous process, but the optimum temperature for germination is considerably above the optimum for after-ripening. Seeds completely after-ripened at 5° C. are stimulated to very rapid growth when placed at higher temperatures. On the other hand, if seeds are completely after-ripened and then allowed to desiccate at higher temperatures, seedling vigor is lowered as time progresses, and in several weeks the

embryo fails to respond when placed in favorable germinative conditions. The reason for this loss of vigor is not known. It may be due to the increased respiration, using up the plastic substances essential for the initiation of germination, or to the introduction of some new factor inhibitory to growth. After-ripened seeds placed at $-5°$ C. and kept saturated by packing in snow will retain their initial vigor for a considerable time.

Oxygen pressure

The most favorable oxygen pressure for after-ripening was not studied in detail. Seeds after-ripened in desiccators are under considerably reduced oxygen pressure. The oxygen is soon used up in respiration. Nevertheless, these seeds stored at a low constant temperature will after-ripen quicker than seeds stored out of doors with a good supply of oxygen, but subjected to fluctuating temperatures. Seeds stored in open baskets, but kept saturated at low constant temperatures, will after-ripen sooner than those stored in desiccators, and the resulting seedlings appear to be more vigorous.

Oxidase and peroxidase

Eckerson (11) found an increase in oxidase and peroxidase activity accompanying after-ripening in the haw. In the peach Crocker and Harrington (6) found no increase in oxidase activity in the after-ripening seeds when ordinary chromogens or the Bunzel methods were used, but the pulp of the after-ripened seeds exposed to air shows a more rapid oxidation of its own chromogens. In the sugar maple there is a slight increase in peroxidase activity accompanying after-ripening, being more pronounced in the hypocotyl. No oxidase could be detected in dormant or after-ripened seeds when guaiaconic acid or benzidine was used as a chromogen.

Catalase

One of the most consistent phenomena accompanying the after-ripening of this type of embryo is the increase in catalase activity. This increase is continuous, increasing manyfold during the early stages of germination. Eckerson (11) found that catalase activity increased in the haw with after-ripening. In

Tilia Rose (22) also found a noticeable increase in catalase activity accompanying after-ripening. Crocker and Harrington conclude that "seeds that after-ripen in a germinator at low temperatures (commercial layering), in which the dormancy of the embryo is self imposed and the embryo experiences fundamental time-requiring changes for after-ripening, show a great increase in catalase activity with after-ripening (*Crataegus*, *Tilia*, *Prunus*)."

Catalase determinations were made upon the dormant and after-ripened seeds and upon the seedlings at various stages of germination. In all cases the integuments were removed and a definite number rather than a definite weight of seeds was used. The material was weighed and samples were run as described for the soft maple. The after-ripened seeds and also the seedlings used were after-ripened and germinated in the dark at 10° C. Table VI demonstrates the great increase in catalase activity accompanying after-ripening and germination in seeds of the sugar maple.

TABLE VI

Stage	cc. of O_2 liberated by 1 seed or seedling in		cc. of O_2 liberated per gm. of dry weight
	5 minutes	10 minutes	10 minutes
Dormant	23.4	31.1	754
After-ripened	33.7	39.3	1117
Seedlings with 1 cm. radicle	31.0	37.0	1058
" " 2 " "	51.0	60.4	1716
" " 3 " "	87.2	98.4	2235
" " 4 " "	99.7	114.0	2230
" " 5 " "	89.2	107.0	2786
" " 6 " "	113.2	130.0	4481
" " 7 " "	125.0	142.5	4440

An increase in catalase activity is evident in both cotyledons and hypocotyl. Seeds germinated at higher temperatures also gave slightly increased catalase activity when taken at the same stage of development. Seedlings with radicles 1 cm. long were used to determine the relative catalase activity of the different parts. One-tenth gram (wet weight) of radicles, cotyledons, and integuments liberated in 10 minutes 95, 43, and 5.1 cc. of oxygen respectively. The hypocotyl, which is the most actively growing

organ at this time, gives by far the greatest catalase activity. The storage organs (cotyledons) give considerable catalase activity. The inert structures (integuments) give very low catalase activity. The difference here would be still more striking if calculated as percentage of dry weight. CROCKER and HARRINGTON find the catalase activity of wheat embryo 28–29 times that of the endosperm. The same investigators find that in grass seeds in general the physiologically inactive organs show only a small fraction of the catalase activity shown by the embryo.

Dry dormant seeds stored in the laboratory were used to determine the Q_{10} for catalase activity at temperatures ranging from 10° C. to 50° C. Seeds were ground very fine and rubbed through a 100-mesh sieve. One-tenth gram samples were used for determinations. Ten cc. of dioxygen, 10 cc. of water, and a small excess of $CaCO_3$ were added to the meal. Table VII shows the Q_{10} value for catalase activity.

TABLE VII

TEMPERATURE	Q_{10} FOR		
	1 minute	5 minutes	10 minutes
10–20° C.	1.4	1.3	1.3
20–30° C.	1.3	1.2	1.1
30–40° C.	0.1	0.9	0.8
40–50° C.	0.8	0.6	0.5

In no case does the van't Hoff law, which calls for an increase of 2–3-fold for every 10° C. rise in temperature, hold. The time consumed in heating the sample to the higher temperature introduces considerable error. The time required for complete destruction of catalase activity at any given temperature was not determined. There was still some catalase activity at temperatures slightly above 50° C. APPLEMAN (2) found the catalase activity in potato tubers to be entirely destroyed at 50° C. Between c° C. and 10° C. he finds the Q_{10} for catalase activity to be 1.5. From 10° C. to 40° C. he gets lower Q_{10} values for potato catalase than was given by the catalase of the sugar maple.

Chemical analysis

Samples were analyzed as in river maple, with slight modifications to suit the material. One-tenth gram of $CaCO_3$ was added to samples at the time of collection. Figures in the tables represent averages from several samples. Dormant seeds had made no progress in after-ripening. It is almost impossible to choose seeds for the after-ripened samples that are known to be completely after-ripened. The only criterion for completion of after-ripening is germination. The seeds in the after-ripened samples vary from completely after-ripened ones to seeds probably within a week or 10 days of complete after-ripening.

TABLE VIII

Stage	Sugar calculated as percentage to total dry weight		
	Free reducing sugar	Sucrose (as invert sugar)	Polysaccharides (as glucose)
Dormant	0.06	6.40	5.21
After-ripened	0.67	4.32	4.66
Beginning germination, radicles about 1 cm	1.81	2.36	3.43
Seedlings with 2–3 cm. radicle (with integuments)	1.13	1.80	5.91
Seedlings with 5–6 cm. radicles (integuments shed)	0.06	2.62	5.43

The protein content of the seeds is exceptionally high. The seeds contain 7.17 per cent of nitrogen or approximately 44.8 per cent protein, calculated on a dry weight basis. The embryo itself contains almost 50 per cent of protein. The nitrogen multiplied by the factor 6.25 was used to indicate the amount of protein present. The seeds contain about 17 per cent of ether extract and 11.5 per cent of total sugars. The ash percentage is relatively high, 5.87 per cent of dry weight, while 0.91 per cent of the total dry weight is phosphorus. Only a trace of free reducing sugar is present in the dormant seeds, but sucrose or sucrose-like sugars are present in considerable amounts. Table VIII shows the relative amounts of various sugars at time of dormancy, approximately complete after-ripening, and early stages of germination.

Accompanying after-ripening there is a considerable increase in free reducing sugars. Free reducing sugar reaches a maximum at the beginning of germination, and then diminishes as germination progresses. There is, no doubt, a considerable amount of sugar used up in respiration during the long after-ripening period in the germinator even at temperatures as low as 5° C. Whether the appearance of considerable amounts of free reducing sugars is merely correlated with after-ripening or is essential for the completion of after-ripening is not known. The formation of free sugars may be favored by cool uniform temperatures and high state of hydration of the embryo.

TABLE IX

Stage	Kjeldahl nitrogen as percentage of total dry weight in		
	F_1	F_2	F_3
Dormant	0.03	1.58	5.56
After-ripened	0.03	1.48	5.59
Beginning germination, radicle about 1 cm	0.03	1.63	5.29
Seedlings with 2–3 cm. radicle (with integuments)	0.03	2.37	4.73
Seedlings with 5–6 cm. radicle (integuments shed)	?	3.15	4.94

Seedlings with radicles 2–3 cm. long show an increase in polysaccharides, but a decrease in free reducing and sucrose or sucrose-like sugars. Correlated with this increase in polysaccharides is a considerable reduction in percentage of fat. The percentage of ether extract drops from about 17 per cent in the dormant and after-ripened seeds to slightly less than 14 per cent in the seedling with a radicle 2–3 cm. long. The fats in the early stages of germination are probably converted into sugar or sugar-like materials, as found in the haw by ECKERSON (11), in the sunflower by MILLER (19), and in the castor bean by DELEANO (9).

With germination there is the usual increase of the more soluble nitrogen of F_2. There is no significant change in relative nitrogen value of the dormant and after-ripened seeds. Table IX shows the relative amounts of nitrogen in the various fractions at different stages of the seeds and seedlings.

Respiration

A detailed study of respiration of the after-ripening seeds at the lower temperatures may help to interpret the metabolic activity accompanying after-ripening. Little work has been done on this phase up to the present time. Preliminary tests show very little respiration taking place in dormant air-dry seeds. When these seeds are soaked for 48 hours, however, and then transferred to the respirometer, the respiratory intensity jumps to approximately the same level as that of fully after-ripened seeds. Sufficient data have not been obtained to justify a full discussion of the correlation between after-ripening and respiration.

Hydrogen ion concentration

The gas chain method described by MICHAELIS (18) was used to determine the hydrogen ion concentration. Two embryos were used in each case. They were ground for 2 minutes with a small amount of pure quartz sand and 1 cc. of distilled water, and 5 cc. of distilled water was then added. This solution becomes more alkaline the longer it stands, so several readings were taken immediately and the average of these used. In both the dormant and after-ripened embryo we find a distinctly basic condition. The average of several samples shows a P_H value of 8.335 in the dormant seeds and a P_H value of 7.909 in the after-ripened seeds. Both are distinctly on the basic side of the neutral point. The hypocotyls of the dormant seeds gave a P_H value of 9.048, while that of the germinating seedlings with a 1 cm. hypocotyl gave a P_H value of 9.055. Seeds that had just started to germinate were used in the latter case, to be sure that the period of after-ripening had been completed. ECKERSON (11) found increased acidity in the hypocotyl of the haw with after-ripening. In working with *Tilia americana* ROSE (22) found increased hydrogen ion concentration with after-ripening. In the sugar maple the embryo is always basic, although the hydrogen ion may increase in concentration in the embryo when it after-ripens.

Discussion

To the present time little work has been done upon seeds that show in general the same type of behavior as found in the river

maple. Numerous observers have reported cases of seeds dying when subjected to atmospheric conditions for a short period of time. As to just what factors operate with desiccation to cause lowering of seedling vigor and early death we are still entirely ignorant. In the river maple temperature does not appear to determine the critical percentage of water loss. Death occurs at all ordinary temperatures (0–35° C.) when the percentage of water in the seeds has reached 30–34 per cent. Whether or not this will hold in general for other seeds of this type will not be known until considerably more species have been studied. In the desiccated seeds we find a noticeable increase in permeability, indicated by a large amount of sugar appearing in the substratum when placed in the germinator. The sugar makes an excellent medium for growth of bacteria and fungi, and in a few days the entire seed is completely decomposed. The fungi appear to be unable to attack potentially vigorous seeds. Whether increased permeability is the cause or the result of death is not known. Desiccation may coagulate or denature the protoplasmic proteins, increasing permeability and subsequent leaching, allowing an inroad for parasitic organisms. This type of seed stands in marked contrast to that type of seed which retains its viability best when stored in an air-dry condition. DUVEL (10) even recommends drying the majority of seeds in a vacuum or over sulphuric acid to insure good preservation. In fact, many seeds can be dried to constant weight without lowering viability or seedling vigor. KIDD (15) states: "In the case of certain rapidly deteriorating seeds (*Hevea brasiliensis*) the carbon dioxide naturally produced by respiration of the seeds in a closed flask rose to 40 per cent, and the pressure of this was found to be accompanied by a marked prolongation of vitality in the seeds. This prolonged vitality was far in excess of that reached with the present commercial method of packing these short-lived seeds for export." Where there is a rapid oxidation of food material due to high respiration, there is no doubt that narcotizing the embryo would result in greatly reduced metabolic activity. Whether or not high embryo vigor can be maintained in the river maple by narcotizing still remains to be determined. Storage at 0° C. over water, however, provides an excellent condition for the seeds of river maple.

Recent studies have thrown considerable light upon the behavior of seeds that require a definite time under certain favorable conditions to after-ripen a morphologically mature embryo. The major portion of the work up to the present time has been done upon various members of the Rosaceae. No doubt seeds of this general behavior exist in many more of our plant families, especially among the uncultivated forms. Not until more work has been done upon a wider range of plants will it be known just how widespread this phenomenon is. The few species studied thus far by various investigators show remarkable similarity of behavior in several features accompanying after-ripening. There are five more or less specific changes, according to CROCKER and HARRINGTON (6), which are quite conspicuous in the constant way which they seem to accompany after-ripening in seeds of this type: (1) rise in vigor of seedling, (2) increase in amount of water absorbed, (3) increase in total acidity, (4) increase in catalase, and (5) oxidase activity.

When after-ripening is accomplished under the most favorable conditions of oxygen pressure, water relations, and temperature, seedling vigor is in all cases at its maximum. In the sugar maple, at least, seedling vigor can be judged only during the first stages of germination after the completion of the period of after-ripening. After-ripening, however, may complete itself under conditions not favorable for the greatest expression of seedling vigor.

ROSE found slight increase in acidity accompanying after-ripening in the seeds of *Tilia*. This was correlated with greater water holding capacity. In the haw (11) delayed germination of the embryo has been found to be due to a dormant hypocotyl. In the dormant seed this organ is slightly alkaline or neutral, but with after-ripening the hypocotyl becomes distinctly acid. Accompanying this increased acidity there is increased water holding capacity of the hypocotyl, along with increased activity of the enzymes. Here the hydrophilous colloids have a greater water holding capacity in a slightly acid medium. When the entire seed of the haw is considered, however, we find a slightly higher water holding capacity in the dormant than in the after-ripened seed. In the sugar maple the water holding power of the hypocotyl only was not determined. Considering the hydrogen ion concentration

found in the hypocotyl of the dormant and after-ripened seeds, one would hardly expect to find a change in the water holding capacity of the hydrophilous colloids. Determinations on the water content of entire seeds stored in favorable after-ripening conditions show that there is no change in the water holding capacity of the seeds as a whole.

One of the most consistent phenomena accompanying after-ripening in this type of embryo is the great increase of catalase activity. This appears to be an accompanying feature of more than ordinary importance. A large number of investigators in various branches of animal and plant physiology attempt to correlate catalase activity with metabolic activity in general. BURGE (3), by increasing the work of certain fowl muscles and consequently the respiratory and metabolic activity, has made the catalase activity increase enormously. In the castor bean DELEANO (9) found a rapid increase in catalase activity at the beginning of germination. A great increase in catalase activity accompanied germination in the sugar and river maples. In the fully imbibed seed of Johnson grass, CROCKER and HARRINGTON (6) found catalase activity paralleling respiration. This did not hold for seeds of the amaranth, however. In the potato, APPLEMAN (1) found respiratory and catalase activity closely accompanying each other. ECKERSON (11) found an increase in the catalase activity with after-ripening in the haw. An increase in catalase activity with after-ripening has also been reported for *Tilia americana* (22). In the sugar maple there was a 66 per cent catalase activity increase in the after-ripened seeds over that of the dormant seeds. Just how closely catalase activity and respiration parallel each other during the course of after-ripening has not yet been determined. From evidence at hand showing the almost universal correlation of these two phenomena we might reasonably expect to find respiration increase noticeably during the process of after-ripening. Respiratory activity should be determined continually throughout the entire period of after-ripening at the temperature and water relations most favorable for after-ripening. Preliminary respiratory determinations reported in this paper are not conclusive. The seeds were transferred from 5° C. to the 20° C. oven. This change

in temperature no doubt introduces changes which may possibly mask the real condition at the lower temperature.

Accompanying after-ripening in the sugar maple is an increase in the amount of free reducing sugars. Just how generally this occurs in this type of embryo is still unknown. Whether increase in amount of free reducing sugar is essential for the completion of after-ripening is problematical. Dormancy is probably due to a temporary suppression in the development of one factor or a group of factors essential for the normal functioning of the embryo in germination. It is impossible to select any one factor as the cause of dormancy in the embryo of the sugar maple at the present time. Whether any certain observed change in the embryo accompanying after-ripening is responsible for bringing dormancy to a close, or whether this change results merely from the conditions to which the embryo has been subjected, remains a question.

Summary

River maple

1. Seeds lose their viability when the water content is reduced to 30–34 per cent.

2. Temperature seems to play no part in determining the critical point of water loss. Higher temperatures only hasten the rate at which the point of desiccation is attained.

3. Respiratory activity in the desiccating seeds at 25° C. first decreases slightly, then rises to a maximum, then gradually falls to zero as desiccation progresses.

4. After a slight initial increase, catalase activity gradually decreases in the desiccating seeds. Catalase activity increases enormously during the early stages of germination.

5. Seeds of a river maple may be kept in a vigorous viable condition for a considerable period of time at low temperatures (0° C.) stored over water.

6. There is a gradual decrease in peroxidase activity accompanying desiccation.

Sugar maple

1. Seeds after-ripen best at temperatures near 5° C., with a good supply of oxygen and moisture.

2. With after-ripening the seeds show a considerable increase in free reducing sugars.

3. Catalase activity increases greatly with after-ripening and germination; there is also a slight increase in peroxidase activity.

4. Both the dormant and after-ripened seeds have a reaction that is distinctly alkaline; this holds for the hypocotyl as well as for the entire embryo.

5. Fully after-ripened seeds will remain in this condition for a long time if kept moist at $-5°$ C.

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POLYEMBRYONY AMONG ABIETINEAE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 261

(WITH FIFTEEN FIGURES)

JOHN T. BUCHHOLZ

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(WITH FIFTEEN FIGURES)

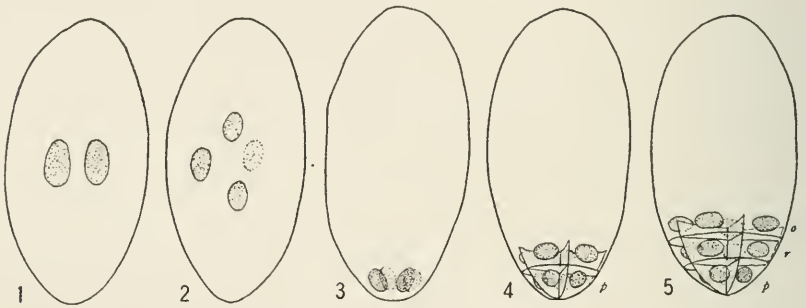
Polyembryony among conifers is of two kinds: cleavage polyembryony, in which a single fertilized egg gives rise to many embryos; and the simple polyembryony, which is due to plurality of archegonia. This latter form is encountered wherever there are several eggs that may be fertilized, and therefore is found among all gymnosperms. The fact that polyembryony was found in both the pines and the cycads, and was due to plurality of "corpuscula" or "areolae" (archegonia) in both instances, was one of the arguments presented by BROWN (1, 2) as early as 1826 as showing a fundamental relationship between these two groups.

A form like *Pinus*, which has cleavage polyembryony, usually has several eggs fertilized also, and therefore combines both forms of polyembryony. Since each zygote in *Pinus* usually gives rise to a system of 8 embryos, there may be as many embryos as 8 times the number of fertilized eggs. If all 6 of the archegonia of some species were fertilized, 48 embryos might be produced, but 4 is the maximum number of embryo systems that have actually been found, and even then many of the embryos disappear very early, some of the rosette embryos being aborted without division of the embryo initial cell.

In discussing polyembryony, it is necessary to consider briefly the pine proembryo stages, shown in the accompanying figures. The writer's interpretation of the facts brought out by various investigators, together with his own studies, would describe the initial steps in the development of the pine embryo as follows.

The zygote begins development with free nuclear divisions (figs. 1–3). When 4 free nuclei have been formed they descend to the bottom of the egg, and there undergo another free nuclear division, after which the primary embryo initial group of cells (*p*) is

cut off by complete walls from the rest of the cytoplasm of the egg. Each cell of this tier constitutes an initial cell to one of the 4 primary embryos. The tier above it is not completely walled, and therefore undergoes another free nuclear division, organizing the second tier of completely walled cells (*r*), the rosette tier, a group of initial cells of the rosette embryos. The open tier of free nuclei (*o*) which remain above this undergo no further division and soon disintegrate. When these 3 tiers of 8 walled cells and 4 free nuclei have formed, as in fig. 5, the organization stage of the proembryo is concluded, for each cell is now ready to produce its own distinct embryo, although the 4 cells of the primary embryo initial tier (*p*) continue their further development in unison.

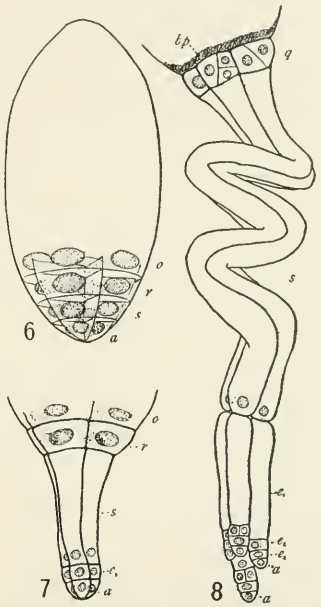


FIGS. 1–5.—Steps in development of proembryo in *Pinus*, diagrammatic reconstructions from serial sections and published figures: *p*, tier of primary embryo initial cells; *r*, tier of rosette cells, initial cells of rosette embryos; *o*, upper open tier of cells; normally tiers *r* and *o* come from division (free nuclear) of upper tier of fig. 4.

From each of these 8 completely walled embryo initials (fig. 5) an embryo develops by means of an apical cell, this cell functioning first as a hemispherical apical cell of one cutting face, and later as a semi-pyramidal cell of 3 cutting faces, in a manner described in greater detail elsewhere (3). It may be added that this apical cell persists until an embryo mass of about 500 cells has been formed, after which it is replaced by the meristematic group of cells found in the older conifer stem tip. This apical cell is a primitive feature in which conifers recapitulate their fern phylogeny.

THE EARLY EMBRYO OF PINUS.—The cells (*p*) of the embryo initial undergo simultaneous division, in which their first apical

cell segments (*s*), the primary suspensor cells, are cut off. This group constitutes what has generally been recognized as the suspensor tier of the 16-celled stage (cf. figs. 5, 6). Next the suspensor cells (*s*) elongate and thrust the embryonal tier of apical cells into the pocket which the digestive enzymes of the eggs and embryos have corroded within the gametophyte, the 4 embryo units separating and their apical cells (*a*) continuing to give rise to segments (e_1, e_2, etc.), which elongate and add to the suspensor.



FIGS. 6–8.—Stages in development of early embryo in *Pinus*: *a*, apical cells; *s*, primary suspensor cells; *r*, rosette cells, which give rise to *q*, rosette embryos (latter usually develop later than in stage of embryo shown); e_1, e_2, etc., embryonal tube initial cells and embryonal tubes, which elongate and add to suspensor; diagrammatic reconstructions.

Soon the rosette group of initials divides and the development of the rosette embryos is begun (*q*, fig. 8). It will be seen, therefore, that not only do these 8 embryos per zygote all result from free nuclear cleavages, but the several embryos develop independently from the time the first walls are organized. The primary embryos develop without interruption from their initials, while the rosette embryos are delayed, developing somewhat later, on an average, than is indicated in fig. 8. In the hundreds of instances that have been examined in my investigations of various pines, none were found where the 4 primary embryos were combined to produce a single embryo, nor were any cases found where one of the primary embryos was further split up to give rise to 2 or more embryos.

In the competition which ensues, the rosette embryos play a very subordinate rôle, owing to their unfavorable position and delayed development. Among the 4 primary embryos, the competitive process elects one embryo from the complex, nearly always the embryo which develops the longest suspensor, pushing it ahead of

its competitors. Embryonic vigor in producing a long suspensor is the outstanding factor which decides upon the successful embryo. The mass of embryonal tubes which elongate from the base of the embryo, as this and the suspensor become more massive, doubtless assist the successful embryo in checking the others. Usually it is the embryo foremost in position which is successful in developing to maturity, but sometimes the second one in position becomes massive more rapidly and assumes the leading rôle, by choking out the smaller terminal one. Not only must an embryo have a rapidly developing suspensor, but it must also become many-celled and massive more quickly than any of the competing embryos.

Vigorous suspensors have been the basis of selection among the embryos of gymnosperms for so long a period that this organ has become a large and extensively developed structure, many times larger than would be necessary without this embryonic competition. This is true whether the competing embryos come from the same egg, as in cleavage polyembryony, or the selection occurs between neighboring zygotes, as among cycads. The remarkably long suspensor found in nearly all gymnosperms has always been a noteworthy feature of this group.

Investigation

OTHER PINE SPECIES.—The result of a further investigation of the embryo development in various species of pines confirmed the account as announced for *Pinus* (3). The additional work done on *Pinus Strobus*, *P. ponderosa*, *P. edule*, and *P. resinosa*, as well as a further examination of *P. Laricio*, *P. Banksiana*, and *P. sylvestris*, makes it practically certain that cleavage polyembryony, the apical cell development, and the rosette embryos are found quite constantly among all members of this genus.

It might be noted that *Pinus sylvestris* seems to have a marked tendency to produce shorter suspensor cells and embryonal tubes than *P. Banksiana*, which was taken as the type for the previous investigation. In *P. Laricio* the 4 primary embryo units frequently do not split apart until the primary suspensor cells have stretched to about half their final length and the first embryonal tubes are beginning to elongate. Indeed, when some of these earlier stages

were examined, the writer's prediction was that in this species, at least occasionally, the usual separation into 4 primary embryos did not occur, but hundreds of embryos dissected out in slightly later stages (several days older) of material from the same source failed to reveal even one case without the usual cleavage polyembryony.

The rosette embryos of *Pinus Laricio* are very clear. In many cases they have suspensors which elongate distinctly, and were it not for the fact that the dissections clearly show their relation to the basal plate (*bp*), these rosette embryos would in some instances very easily be confused with the primary embryos. On the whole, the embryos of *P. Laricio* furnish probably the most satisfactory type for use in laboratory instruction, both on account of their clearness in displaying the rosette embryos, and their large size, which makes them easier to dissect.

Abietineae.—The other genera of Abietineae that were dissected and examined are *Cedrus libani*, *Tsuga canadensis*, *Abies balsamea*, *Picea mariana*, *Picea excelsa*, *Larix europea*, and *Pseudotsuga taxifolia*, the species investigated representing 7 out of the 9 genera of the Abietineae.

Method and material.—The technique was that of dissection described in detail in the writer's work on *Pinus*. No modifications of these methods were found necessary, but perhaps it should be repeated that the living material is indispensable for some species. A study of preserved material is possible, but it is not so satisfactory. The embryos may be killed and preserved indefinitely, however, after they have been removed by the methods described. The proembryo stages must be studied by the well known methods for making serial sections. The writer is indebted to the following for the material used during the summer of 1917: W. G. Waterman for material of *Abies* and *Tsuga* from Frankfort, Michigan; S. D. Magers for collections of *Abies balsamea* and *Picea mariana* from Marquette, Michigan; D. Hill Nursery Company, of Dundee, Illinois, for material of *Pseudotsuga*, *Larix*, and *Tsuga canadensis*, collected on their grounds. Very satisfactory material of *Pseudotsuga taxifolia* was supplied by the Friday Harbor Marine Station of Puget Sound. During June and July C. T. Hilmers supplied

weekly collections of the material growing on the University Farm near Lincoln, Nebraska, as follows: *Picea excelsa*, *Pseudotsuga taxifolia*, *Pinus ponderosa*, *P. sylvestris*, *P. Laricio*, and *P. Strobus*. In addition to this, the writer made many trips to various places in the vicinity of Chicago to secure material of some of these same species. During the summer of 1918, W. W. ROBBINS supplied a collection of *Pseudotsuga taxifolia* from near Fort Collins, Colorado, and arranged for a collection of *Pinus edule* from Cortez, Colorado; and E. J. KRAUS made several collections of the cones of *Cedrus libani* from the grounds of the Oregon Agricultural College, Corvallis, which reached the writer in excellent condition.

Cedrus has almost the same early embryogeny as *Pinus*. The primary embryos, however, do not separate until some time after the suspensor cells and first embryonal tubes have both elongated, and therefore cling together very much longer than in any species of *Pinus* that was investigated. In all the slightly older stages the embryo units had separated, indicating that cleavage polyembryony is likewise a constant feature in *Cedrus*. An apical cell stage seems to exist in this genus, and rosette embryos usually occur, somewhat less developed than in the average pine. The older suspensor cells collapse soon after separation of the primary embryo units.

Tsuga canadensis also resembles *Pinus* very much in its embryogeny. In this species the embryo units separate into the 4 primary embryos, yet they cling together longer than in any pine, apparently about as long as in *Cedrus*. Cleavage polyembryony occurs regularly. This conclusion is based upon the careful dissection and examination of the embryos of about 40 ovules of a more advanced stage, among which no exceptions were found.

Save for their difference in size, *Tsuga*, *Cedrus*, and *Pinus* appear very similar in the first stages of suspensor formation. In *Tsuga*, however, the rosette cells are very ephemeral; they were not found to divide before the collapse and disintegration of their contents, apparently giving no rosette embryos. The suspensor cells also collapse very soon in *Tsuga*, leaving only a shred of tissue which connects the shriveled rosette to the embryo system below. As in *Pinus*, the early embryos develop by means of an apical cell.

There are from two to four archegonia present in *Tsuga*, and in the material studied one or two embryo systems was the usual number found. The cones were very poorly pollinated, and doubtless the normal maximum number did not occur. Polyembryony, although extensive, is much less pronounced than in *Pinus*, for in addition to the small number of archegonia, there are no functioning rosette embryos.

In *Abies* the normal product of a fertilized egg is a single embryo. The group of rosette cells is present, and in a few rare instances a divided rosette cell and a more advanced rosette embryo were found. This, as well as the fact that cleavage polyembryony was also observed in a few cases, shows that this genus stands next to *Cedrus* and *Tsuga* in its similarity to *Pinus*.

The apical cell stage is doubtless eliminated from the beginning, for when under normal conditions all of the lower tier of cells combine to produce a single embryo, the terminal cells together are responsible for producing the tissue. It appears also from an examination of some of the early embryos that these 4 terminal cells of the apical group do not always contribute equally to the cell mass, for one of these 4 terminal cells may frequently be found decidedly more prolific than the others. Normal apical cell growth, however, is not possible unless cleavage polyembryony occurs, as it rarely does.

The suspensor cells and upper embryonal tubes of the secondary suspensor collapse very soon after elongation. The basal plate (*bp*), a deposit formed within the egg over the rosette cells, is very thick and frequently obstructs a clear view of the rosette cells, which also collapse early, unless a rosette embryo happens to develop.

The material of *Picea* was somewhat limited. The cones that could be secured of *P. mariana* were younger than the fertilization stage, and a later collection was too old for a satisfactory study of the early embryo. A number of twigs bearing cones from the first collection were kept in a tin box in the laboratory for more than a week, and at the end of this time they were found to contain embryos in the desirable stages. The *P. excelsa* cones were very poorly pollinated, and only a few good embryos were secured from

this species. A study of this material makes it clear that cleavage polyembryony does not occur, but each archegonium produces only a single embryo. The group of rosette cells is present, but no divisions were found within these cells producing rosette embryos, as they do occasionally in *Abies*. *Picea*, therefore, is a step farther removed from *Pinus* in having eliminated all traces of cleavage polyembryony and rosette embryos, except the tier of rosette cells.

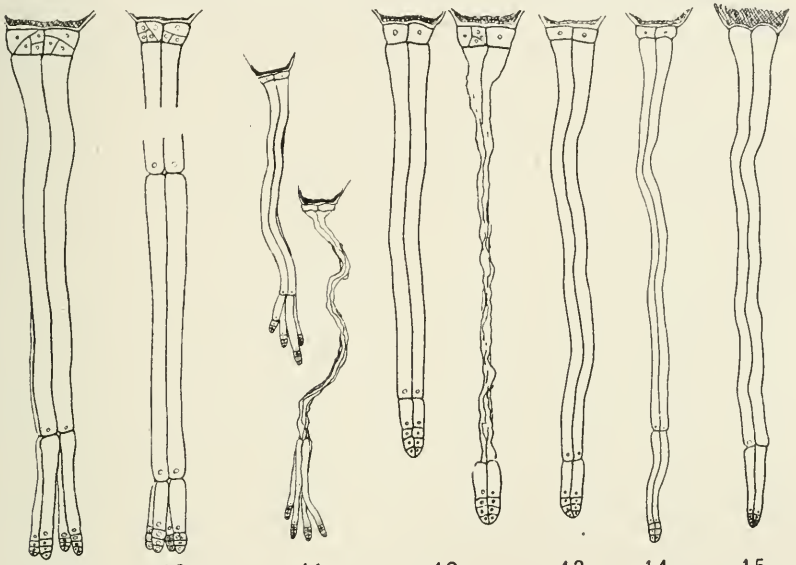
Although the available material of *Larix* was also somewhat limited, several outstanding features may be described with certainty. Like *Picea* and *Abies*, only one embryo is produced per archegonium. Except for the different appearance in size and proportion, the embryo of *Larix* is very similar to that of *Picea*. The 4 collateral primary suspensor cells become very long and slender, without the abrupt twists or turns found in the pine suspensor, and the secondary additions of the suspensor have similar characteristics. The older divisions of the suspensor collapse as the newer embryonal tubes elongate from the base of the embryo. A group of rosette cells is present, but these collapse without forming embryos, and the basal plates are again large, obstructing a good view of the former in many cases.

Pseudotsuga furnishes a rather interesting variation from the embryos already described. This form is like *Picea* and *Larix* in producing only one embryo from each egg. It has no rosette cell, but the uppermost tier of walled cells elongates to form the suspensor, a condition shown in less than 5 per cent of the pine embryos (*Pinus Banksiana*). This occurs as a regular feature in *Thuja* (12) and many other conifers. As the suspensor elongates, the contents of the archegonia shrink and harden, and persist as flattened, deeply stained structures attached to the upper ends of the transparent suspensors. A very thick layer of protoplasm or other substance, in the position which corresponds to the basal plate, stains more deeply than the remaining regions of the withered archegonia. Although cleavage polyembryony does not occur, a larger number of embryos is produced than in *Abies*, *Larix*, or *Picea*. This is due to the existence of a larger number of archegonia, which range from 5 to 8. The suspensor cells do not collapse early, as in *Larix* and *Abies*, and although the embryos were never found splitting into

separate units, the suspensor cells back of the embryo become easily separated from each other.

Discussion

It will be seen that among the 7 genera of the Abietineae examined, the last three do not possess cleavage polyembryony even as an occasional feature, while in *Abies* it occurs only in rare instances. Likewise the rosette embryos occur normally in *Pinus*



FIGS. 9–15.—Embryos of 7 genera of Abietineae, showing intergrading series with cleavage polyembryony on the one hand (figs. 9–11) and its absence on the other (figs. 12-15); rosette embryos in *Pinus*, *Cedrus*, and occasionally *Abies;* diagrams not drawn to scale.

and *Cedrus*, and only rarely in *Abies*, while none of the other forms shows them even occasionally. *Cedrus* and *Tsuga* are most like *Pinus* in possessing cleavage polyembryony as a constant feature, but in the latter the rosette cells do not produce rosette embryos. Rosette cells, even though they produce no embryos, as in *Tsuga*, *Larix*, and *Picea*, are clearly homologous with these embryo initials in *Pinus* and *Cedrus*, and represent vestigial structures wherever they are present. Figs. 9–15 illustrate these differences. We have

here a very interesting intergrading series, with *Pinus* at one end and *Pseudotsuga* at the other. There seem to be but two alternatives; either the *Picea* or *Pseudotsuga* type of embryo has given rise to the *Pinus* type with cleavage polyembryony, or the *Picea* embryo is composite in its origin, being made up of the fused or combined elements that produce the many cleavage embryos in *Pinus*.

The writer believes that the pine embryo with its cleavage polyembryony is the primitive type, and the following are among the reasons for this conclusion. The pine embryo combines with cleavage polyembryony the apical cell, a primitive character, which clearly recapitulates its semi-pyramidal predecessor at the stem tip of the fern. To assume that cleavage polyembryony is a derived feature would take away all phylogenetic significance from this structure, for the *Picea* and *Pseudotsuga* type of embryo have no apical cell. The apical cell could hardly be considered an accidental result of the splitting of a *Picea*-like embryo. This conception might be entertained if the terminal cell began to display apical cell characteristics only after separation of the embryos, but a true apical cell has been shown to exist from the embryo initial stage, from the time the first walls appear in the proembryo.

The apical cell is present in the adult ferns and in the first stages of the pine embryo; it is absent in all adult gymnosperms and likewise in angiosperms. This structure has been eliminated in passing from the lower to the higher vascular plants, and in *Picea*, *Larix*, and *Pseudotsuga* the apical cell is entirely eliminated from the beginning of the life history. The embryo development in this group shows how the apical cell was lost in the evolution of the Abietineae.

Another reason why the *Pinus* embryo must be considered the more primitive type arises from the study of the rosette embryos. In the *Picea* embryo are found the vestigial rosette cells, which never divide, but are clearly homologous with the rosette embryo initials in the pine. Even in the pine these rosette embryos are vestigial, but since these rudimentary structures are well developed in the latter, one would infer that the *Pinus* type represents the more primitive condition.

Another point in favor of the view that cleavage polyembryony is a primitive feature is the fact that *Pinus* is known to be very old historically. This genus has come to be regarded by paleobotanists as one of the very oldest conifers (6). On the other hand, JEFFREY (9, 10) has reached this same conclusion on the basis of anatomy.

An additional argument that cleavage polyembryony is primitive comes from a consideration of the relation that the pine embryo holds to the known steps in the embryo development of other conifers. There are several lines of evolution which have arisen from a primitive type of embryo like *Pinus*. One of these is the abietineous evolution shown in this investigation, the series beginning with *Pinus* and culminating in *Pseudotsuga*. Another evolutionary series begins with *Pinus*, involves some of the Cupressineae and Taxodineae, and culminates in Gnetales, a line in which cleavage polyembryony has been retained. *Ephedra* has a modified form of cleavage polyembryony, which associates it with Coniferales on the basis of its embryogeny. Other evolutionary lines may have been derived from the *Pinus* type of embryo, as described elsewhere (3). This is therefore another strong argument that the pine type of embryo is very primitive.

STRASBURGER (18) has reported that *Picea* develops only one embryo per archegonium, and his results are thus verified by this study, but he did not attach any significance to the question of whether or not a separation of the embryos occurs. Other investigators in dealing with the embryos of the Abietineae have likewise failed to make this point clear, and the embryogenies of some genera, such as *Cedrus*, *Tsuga*, *Abies*, and *Larix*, have been partially investigated in proembryo stages only.

The proembryo of *Pinus* has been most extensively studied, described, and figured by CHAMBERLAIN (4), COULTER and CHAMBERLAIN (5), Miss FERGUSON (7), and Miss KILDAHL (11), each investigator adding a few additional stages and details. The facts brought out by these investigators are in harmony with the interpretation given to the proembryo in this paper.

The embryogeny of conifers has not usually been undertaken by morphologists as a distinct problem, but the stages described and

figured were often rather incomplete, being only the by-product of another investigation. In several instances the proembryo of other Abietineae has been described as being the same as *Pinus*, but it is doubtful if all of the investigators verified every step of the embryogeny included in their account. Four tiers of 4 cells (fig. 6) may be produced by several methods of division.

LAWSON (13) describes 4 tiers of 4 cells each for *Pseudotsuga*, but since this species has no rosette group, the exact order of division and the stages corresponding to figs. 4–7 in *Pinus* may not be the same. The writer has not had opportunity to examine the proembryo or the earliest stages of the embryo in this species, but it may be inferred that one of two things happens in the *Pseudotsuga* embryo. Either the lowest tier, shown for *Pinus* in fig. 4*p*, continues to divide to give rise to the additional two tiers of cells, or, more probably, the exact order of division shown in *Pinus* is carried out, and it is the rosette tier which elongates. *Pinus Banksiana* (3) was found with elongated rosette cells in nearly 5 per cent of the cases studied. It is very important, therefore, to know whether the divisions that occur in the proembryo of any species are homologous with those of *Pinus*.

MIYAKE (14), in his study of *Picea*, includes the stages of the proembryo, and fortunately he figured a stage between fig. 4 and fig. 5, also between fig. 5 and fig. 6, which proves that the rosette tier found in this form is identical in origin with that of *Pinus*, and the rosettes of these two species are therefore distinctly homologous.

Tsuga and *Abies* probably have proembryos identical with *Pinus*, in view of the results shown for *Picea*. Only a few stages of the proembryo in *Tsuga canadensis* are definitely known. These were figured by MURRILL (17) as essentially the same as *Pinus*, but not illustrated in stages older than fig. 3. *Abies balsamea* was shown by MIYAKE (15) to be practically the same as *Pinus* for the stages up to and including fig. 4. In view of the similarity of *Pinus* and *Cedrus* in their early embryogeny, there can be little doubt that the proembryo of the latter develops in very much the same manner.

Only two genera of the Abietineae have not been investigated in some early stage by the writer. These are *Keteleeria* and *Pseudo-*

larix. The later embryo and other anatomical features of *Keteleeria* are described by HUTCHINSON (8), but the early embryo still remains to be studied. *Pseudolarix* was described by MIYAKE and YASUI (16), whose work shows stages in the embryo similar to figs. 2, 4, and 6, with a figure showing the suspensor cells beginning to elongate. This species has rosette cells and appears more slender, but is otherwise like the average of the Abietineae in the same stage of development before the embryo units separate (if they do). This embryo is not like *Pseudotsuga*, therefore, but probably belongs somewhere in the series (figs. 9–15) between *Tsuga* and *Picea*, the exact position depending upon whether or not cleavage polyembryony occurs, and whether the rosette cells give rise to rosette embryos.

Some taxonomists include *Pseudotsuga* in the same genus with *Tsuga*. The results of this investigation show that, on the basis of the embryogeny at least, there is a fundamental difference between these two forms, which would entitle *Pseudotsuga* to be recognized as a separate genus. The contrasting differences may be summarized as follows. *Tsuga* has cleavage polyembryony and apical cell growth in its life history, while *Pseudotsuga* has none of these features; and while the rosette cells do not produce embryos in *Tsuga*, they are either entirely absent in *Pseudotsuga* or they elongate to form the suspensor and are not recognizable. The latter genus has also 5–8 archegonia, while *Tsuga* usually has a smaller number (2–4).

It should be noted that the difference between the embryo of *Pseudotsuga* and *Tsuga* is greater than that between *Abies*, *Larix*, and *Picea*, and much greater than that between *Pinus* and *Cedrus*. *Cedrus*, on the other hand, shows little in its early embryogeny which would entitle it to a place as a separate genus, but the difference between *Pinus* and *Cedrus* is nearly as great as that between *Larix* and *Picea*.

Summary

1. Although all species of *Pinus* have shown a complete separation of the 4 primary embryos, this feature of cleavage polyembryony is not characteristic of all Abietineae.

2. The cleavages which separate the 8 embryos from each other are the free nuclear divisions of the proembryo. In forms without cleavage polyembryony (*Picea*, and as far as we know concerning other forms), cell divisions homologous with those in *Pinus* occur in the proembryo.

3. The embryos of the Abietineae may be arranged in an intergrading series, with *Pinus* at one end and *Pseudotsuga* at the other, on the basis of the occurrence of cleavage polyembryony, rosette embryos, and the apical cell. The rosette embryos and their vestiges, the rosette cells, are gradually eliminated as we pass from *Pinus* to *Pseudotsuga*.

4. Cleavage polyembryony, rosette embryos, and the apical cell mark a primitive type of embryo development.

5. The embryo development of this group shows how the apical cell was lost in the evolution of the Abietineae.

6. On the basis of embryogeny *Pseudotsuga* is unique and is entitled to rank as a separate genus.

This study was begun at the Hull Botanical Laboratories in the summer of 1917 and is the result of a preliminary study of the embryo material of these conifers. More detailed descriptions of the embryos with illustrations will appear later. The writer takes pleasure in acknowledging his indebtedness to Dr. C. J. CHAMBERLAIN for valuable council in getting this investigation under way.

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CHEMICAL AND PHYSICAL CHANGES DURING GEOTROPIC RESPONSE

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CHEMICAL AND PHYSICAL CHANGES DURING GEOTROPIC RESPONSE

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Introduction

The work reported in this paper was undertaken with the object of making as complete a study as possible of all the chemical and physical processes that might be involved in geotropic response. It was hoped in this way not only to add something to the knowledge of the mechanics of geotropic bending, but also to find some quantitative differences which are associated with the differing rates of growth of the two flanks of the responding organ. It became necessary to drop the work before it was complete. Such results as were obtained are reported in the hope that they may prove of some value to others interested in the problem.

Several studies of one or more of the factors which might be involved have been made. KRAUS (8) found that the water content of the convex flank of organs stimulated geotropically is greater even before bending begins. He also made determinations of reducing sugars and titration acidity on the juice expressed from the organs. He concluded that when a stem capable of negative geotropic response is laid horizontally, increased sugar formation begins at once, and the amount of free acid decreases. This occurs especially on the lower side. There is a movement of water from the upper to the lower side. Thus the concentration of sugar in the juice of the lower side becomes less than in that of the upper.

Miss SCHLEY (9), working with shoots of etiolated *Vicia Faba* seedlings, found rather complex changes in the titration acidity after exposure to gravity. First the concave side was more acid, then the convex, then they became about equal while bending was in progress. After the tip had passed the vertical, the concave side became the more acid, but this difference gradually disappeared. She found the water content somewhat greater on the convex side,

but the samples were taken after bending was practically complete. The percentage of sugar in the convex flank was considerably lower than in the concave, after an exposure of 45 minutes.

In various roots exposed to gravity CZAPEK (3) found an accumulation of intermediate products of oxidation of certain amino acids, due to the presence of an antienzyme which inhibits the normal oxidation of these substances. He found no differences between the upper and lower flanks in this respect. GROTTIAN (7) and GRAFE and LINSBAUER (5) were unable to confirm CZAPEK'S results. The latter workers (6) found that geotropic response causes no differences in catalase activity.

SMALL (10) found increased permeability in the cortical cells of both sides of root tips of *Vicia Faba* when exposed to gravity. The permeability of the lower sides showed a greater increase than that of the upper side.

Changes in the viscosity of the protoplasm during geotropic stimulation were studied by WEBER (11), who found that the viscosity is lessened. ZOLLIHOFER (12) was unable to confirm this result, and states that the method used is subject to large experimental errors.

Experimental work

The first material used in this work was nodes of corn that had completed their growth. The node was cut out, together with about half the internodes above and below, and the sheath removed. The node was then planted horizontally in a bank of moist sand in a box from which light was excluded. This material is especially good because no growth occurs aside from that due to the action of gravity, and because the region which bends in most cases is very clearly defined. After exposure to gravity this region was cut out and divided into upper and lower flanks. There are at least two objections to the use of corn nodes. First, suitable material can be obtained only during a comparatively short time each year. Second, whether a given node will respond to gravity is very uncertain. Some nodes that apparently were healthy and in good condition did not respond at all, and others which showed no evident differences responded readily. This makes practically impossible a study of the period before visible bending begins.

Etiolated *Vicia Faba* seedlings were used for the later work. For the moisture and titration acidity determinations the plants were grown in moist sphagnum in pans. When the shoots had reached a suitable length (6–8 cm.) they were exposed to gravity by setting the pans on edge. In collecting the material, the leaf was removed and the stem divided as accurately as possible into upper and lower flanks. The terminal 3–4 cm. were used. For the other work the plants were grown in moist sawdust in a dark cool room.

TABLE I

MOISTURE AND ACIDITY IN CORN NODES EXPOSED TO GRAVITY

TIME OF EXPOSURE	MOISTURE			ACIDITY IN CC. 0.05 *N* NaOH PER GM. FRESH WEIGHT		
	Upper flank (percentage)	Lower flank (percentage)	Difference (percentage)	Upper flank	Lower flank	Difference
Hours						
3	86.68	87.10	+0.42	0.49	0.48	−0.01
3	87.00	86.83	−0.17	0.47	0.47	.....
6	86.85	86.98	+0.13	0.47	0.53	+0.06
6	87.09	87.18	+0.09	0.51	0.59	+0.08
9	84.97	84.43	−0.54	0.46	0.48	+0.02
9	84.04	85.10	+1.06	0.51	0.54	+0.03
12	83.80	83.19	−0.61	0.49	0.50	+0.01
12	83.10	82.72	−0.38	0.57	0.59	+0.02
15	85.50	84.61	−0.89	0.47	0.46	−0.01
15	84.24	84.26	+0.02	0.47	0.54	+0.07
18	83.50	83.71	+0.21	0.38	0.48	+0.10
18	82.39	82.79	+0.40	0.51	0.55	+0.04
21	82.35	83.40	+1.05	0.61	0.65	+0.04
21	82.31	82.71	+0.40	0.67	0.70	+0.03
24	83.73	82.99	−0.74	0.55	0.57	+0.02
24	82.97	82.90	−0.07	0.58	0.65	+0.07
27	81.39	82.19	+0.80	0.64	0.57	−0.07
27	81.44	82.44	+1.00	0.65	0.76	+0.11

When they had reached a suitable length they were transferred to boards where they were held in place by pieces of cork. The boards were placed upright in a large galvanized iron container, under a spray. They were kept in this position for at least 24 hours, and then exposed to gravity by rotating the board through 90°.

In the determination of moisture the corn nodes were dried to constant weight in vacuo at 80° C. The samples varied in weight from 2 to 5 gm., according to the number and size of the nodes used. Table I gives the results of the series in which the

nodes were exposed to gravity for varying lengths of time, from 3 to 27 hours. In the last column, + is in favor of the convex side and − in favor of the concave. This method of statement is used in all the tables. As already mentioned, corn nodes are not at all uniform in their response to gravity, and because of this fact a second set was run in which nodes that had bent approximately to the degree indicated were used. The results will be found in table II.

TABLE II

MOISTURE AND ACIDITY IN CORN NODES EXPOSED TO GRAVITY

DEGREE OF BENDING	MOISTURE			ACIDITY IN CC. 0.05 *N* NaOH PER GM. FRESH WEIGHT		
	Upper flank (percentage)	Lower flank (percentage)	Difference (percentage)	Upper flank	Lower flank	Difference
0.....	82.41	81.30	−1.11	0.62	0.65	+0.03
5.....	80.27	80.08	−0.19	0.72	0.75	+0.03
5.....	81.19	80.44	−0.75	0.60	0.73	+0.13
5.....	84.68	84.21	−0.47	0.60	0.63	+0.03
10.....	80.42	80.77	+0.35	0.75	0.83	+0.08
10.....	86.04	86.35	+0.31	0.56	0.60	+0.04
15.....	86.31	87.63	+1.32	0.65	0.72	+0.07
20.....	85.13	87.60	+2.47	0.66	0.80	+0.14
25.....	87.12	89.52	+2.40	0.80	0.76	−0.04
25.....	87.52	89.29	+1.77	0.66	0.71	+0.05

Individual differences in moisture content are so great that different samples cannot be compared. It is only possible to compare opposite flanks of the same sample. In general the differences are slight, and in view of the high percentage of moisture present they may not be significant. There are some features of the results which are of interest, however, especially when the two sets are compared. In the time of exposure set the differences are variable, but in general favor the convex side up to 9 hours of exposure. At 12 and 15 hours, when bending is well started, there is a decided difference in favor of the concave side. At 18, 21, and 27 hours the convex side contains much more moisture. The results at 24 hours appear to be anomalous, especially as no corresponding change is found in the other set. In the degree of bending set the differences are more regular and more marked. During the early stages of bending the concave flank contains the more moisture, but

as bending proceeds the convex flank contains more water. The same difference is indicated in the time of exposure set, but because of irregularities in the response of the nodes, it is not so obvious.

The results with *Vicia Faba* shoots are given in table III. The fresh samples weighed about 1 gm. They were dried to constant weight at 100–102° C. The differences are so small and so

TABLE III

MOISTURE AND ACIDITY IN *Vicia Faba* SHOOTS EXPOSED TO GRAVITY

TIME OF EXPOSURE	MOISTURE			ACIDITY IN CC. 0.05 *N* NaOH PER GM. FRESH WEIGHT		
	Upper flank (percentage)	Lower flank (percentage)	Difference (percentage)	Upper flank	Lower flank	Difference
15 minutes...	93.35	93.35	.....	1.40	1.15	−0.25
15 minutes...	93.33	93.33	.....	1.18	1.18	.....
30 minutes...	92.43	92.50	+0.07	1.10	1.15	+0.05
30 minutes...	93.25	93.13	−0.12	0.99	1.05	+0.06
45 minutes...	92.48	91.63	−0.85	1.05	1.12	+0.07
45 minutes...	92.67	92.73	+0.06	1.07	0.94	−0.13
1 hour.....	93.02	93.19	+0.17	1.16	1.19	+0.03
1 hour.....	92.40	92.50	+0.10	1.20	1.16	−0.04
2 hours.....	91.53	91.50	−0.03	1.54	1.58	+0.04
2 hours.....	93.00	93.03	+0.03	1.39	1.37	−0.02
3 hours.....	92.50	92.65	+0.15	1.18	1.20	+0.02
3 hours.....	92.13	92.45	+0.32	1.15	1.10	−0.05
5 hours.....	92.50	92.80	+0.30	1.23	1.17	−0.06
5 hours.....	92.95	93.15	+0.20	1.18	1.10	−0.08
7 hours.....	92.70	92.63	−0.07	1.02	0.91	−0.11
7 hours.....	92.60	92.60	.....	1.13	1.08	−0.05
9 hours.....	92.37	92.70	+0.33	1.13	1.14	+0.01
9 hours.....	92.87	92.93	+0.06	1.25	1.22	−0.03
11 hours.....	92.35	92.00	−0.35	1.13	1.11	−0.02
11 hours.....	92.65	92.65	.....	1.13	1.19	+0.06
13 hours.....	92.69	92.80	+0.11	1.15	1.12	−0.03
13 hours.....	92.87	92.73	−0.14	1.15	1.12	−0.03
17 hours.....	92.97	92.89	−0.08	1.13	1.26	+0.13
17 hours.....	92.25	92.27	+0.02	1.32	1.41	+0.09
21 hours.....	91.60	91.60	.....	1.15	1.11	−0.04
21 hours.....	93.00	93.07	+0.07	0.97	0.95	−0.02

irregular as to be insignificant. At the periods from 1 to 9 hours the convex side seems to contain, in general, a little more moisture, but the differences are too slight to serve as a basis for any conclusions.

For the determination of titration acidity the samples were ground in a mortar with sand which had been treated with HCl and washed free from acid. Fifty cc. of water was added and the mixture titrated to phenolphthalein with 0.05 *N* NaOH. Blanks

were run on the sand and water, and were used to correct the results. There was not enough color in the material to interfere seriously with the phenolphthalein endpoint, but the endpoint is somewhat slow, and, especially with material containing so little acid, the unavoidable errors are apt to cause differences which represent a large percentage of the total titration. The results for corn nodes, calculated as cubic centimeters 0.05 *N* NaOH per gram of fresh material, are given in tables I and II. The differences found between the two flanks are small. The convex side seems quite uniformly to be the more acid.

A few measurements of the hydrogen ion concentration of the press juice of corn nodes which had bent from 5° to 15° were obtained. The measurements were made electrometrically, using a modified form of the Barendrecht electrode. The following P_H values were obtained, that for the upper flank being given first in each case: 4.919, 5.012; 5.136, 5.246; 5.104, 5.198. In these three cases, therefore, the hydrogen ion concentration of the juice of the concave flank was the greater, although, as has been noted, the titration acidity varied quite uniformly in the other direction.

The titration results with *Vicia Faba* are given in table III. The differences are slight and irregular, and do not correspond at all closely with those reported by Miss SCHLEY.

Determinations of hydrogen ion concentration, and electrometric titrations, were made on the press juice of the upper and lower flanks of *Vicia Faba* seedlings that had been exposed to gravity. The material was frozen immediately after collection. A special hand press was used which would remove the juice very completely from samples containing not more than 10 gm. of the fresh material. Five cc. of the juice was taken for the determination. The hydrogen ion concentration was determined immediately, after adding 1 cc. of 0.10 *N* NaOH free from carbonates. This is practically the method used by EMSLANDER (4) in his work with beer. Preliminary experiments showed that the part of the titration curve including these two points is always, for this material, the straight line part of the curve which crosses the neutral line. Usually the two points obtained were on opposite sides of neutrality, so that the cubic centimeters of 0.10 *N* NaOH required to titrate to $P_H = 7.0$

could be calculated by interpolation. In only one case was it necessary to extrapolate.

In table IV are given the P_H values of the press juice, and the cubic centimeters of 0.10 *N* NaOH required to bring 5 cc. of the juice to the neutral point. The results obtained on right and left halves of seedlings not exposed to gravity are given in the last two lines of the table. These results show the magnitude of the differences that might arise from other causes than the action of gravity, such as actual differences between two sides of a plant, and errors in measurement. In a few cases the differences found

TABLE IV

ELECTROMETRIC DETERMINATIONS ON PRESS JUICE OF *Vicia Faba* SHOOTS EXPOSED TO GRAVITY

TIME OF EXPOSURE	HYDROGEN ION EXPONENT			ACIDITY IN CC. 0.10 *N* NaOH PER 5 CC. OF JUICE		
	Upper flank	Lower flank	Difference	Upper flank	Lower flank	Difference
30 minutes...	6.124	6.198	+0.074	0.81	0.77	−0.04
30 minutes...	6.122	6.060	−0.062	0.89	1.05	+0.16
1 hour.....	6.127	6.207	+0.080	0.81	0.71	−0.10
1 hour.....	6.137	6.092	−0.045	0.83	0.92	+0.09
2 hours.....	6.144	6.198	+0.054	0.75	0.77	+0.02
2 hours.....	6.132	6.160	+0.028	0.79	0.75	−0.04
4 hours.....	6.203	6.060	−0.143	0.74	0.81	+0.07
4 hours.....	6.170	6.193	+0.023	0.72	0.75	+0.03
Not exposed.	6.079	6.102	+0.023	0.88	0.82	−0.06
Not exposed.	6.048	6.103	+0.055	0.87	0.79	−0.08

between the flanks of plants acted on by gravity are greater than those in the blank determinations, but where this is the case the differences are not regular in direction.

The plan of the work included as complete a study as possible of the various oxidizing enzymes. Only the catalase had been studied when it became necessary to discontinue the work. Determinations of catalase activity were made by the method of APPLEMAN (1), as modified and used by CROCKER and HARRINGTON (2). Catalase activity decreases from the tip downward, and it is not exactly proportional to the weight of the sample. It was not possible entirely to avoid the errors from both of these sources. The following method was used. After exposure to gravity the

shoot was divided as accurately as possible into upper and lower flanks. A sample was cut from one of the flanks, starting at the tip and going as far as was necessary to obtain exactly 0.200 gm. The other flank was left attached to the plant, and kept in a moist dark place while catalase was determined in the first sample. The second flank was then sampled in the same way as the first, and its catalase content determined. Six plants were used for each period of exposure. The catalase content of the upper flank of three of these was determined first, that of the lower flank of the other three first. The 0.200 gm. sample was ground for 2 minutes in a mortar with sand and a little $CaCO_3$. It was then washed into the apparatus with 15 cc. of water. After the apparatus had reached the temperature of the bath, 5 cc. of H_2O_2 (dioxygen), neutralized with a little $CaCO_3$, was added. Shaking was begun at once, and readings of the volume of oxygen evolved were taken every minute for 10 minutes. The bath was kept at 25° C. and the air temperature did not change significantly during any single set of determinations.

The results given in table V are the cubic centimeters of oxygen evolved in 10 minutes. The average of the results for each of the periods of exposure is in favor of the upper flank, but only in the case of the 1 hour samples were all the results in this direction. In the other sets the individual results vary so widely that no conclusions can be drawn from the averages.

For chemical analysis samples of about 100 gm. fresh weight were used. These were collected in flasks containing 0.5 gm. $CaCO_3$ and sufficient alcohol so that the final concentration was approximately 80 per cent. It was during the collection of the last of these samples that it became necessary to drop the work. In order that the material might not be lost, H. A. Jones consented to complete the collection and carry out the analyses. The writer wishes to express his thanks to Dr. Jones for his kindness in making this addition to the data possible.

The soluble and insoluble portions were separated, and total solids determined in each. Sugars were determined as follows. Aliquots of the extract were evaporated to remove alcohol, taken up with water, and clarified with basic lead acetate. The excess lead

was removed by Na_2SO_4. In the filtrate reducing sugars were determined before and after subjecting it to the standard method for the hydrolysis of sucrose by HCl. The Bertrand titration method was used for determining the amounts of copper reduced. The results are expressed as glucose and sucrose respectively, although it is recognized that other sugars are undoubtedly included. Total nitrogen was determined in both the soluble and insoluble

TABLE V

CATALASE ACTIVITY IN SHOOTS OF *Vicia Faba* EXPOSED TO GRAVITY (EXPRESSED AS CUBIC CENTIMETERS OF OXYGEN LIBERATED BY 0.20 GM. OF MATERIAL)

Time of exposure	Upper flank	Lower flank	Difference
30 minutes	7.15	7.80	+0.65
30 minutes	8.20	8.00	−0.20
30 minutes	8.45	7.50	−0.95
30 minutes	8.40	6.30	−2.10
30 minutes	9.00	10.70	+1.70
30 minutes	7.10	6.70	−0.40
1 hour	9.85	8.65	−1.20
1 hour	9.40	8.20	−1.20
1 hour	12.20	11.40	−0.80
1 hour	10.20	9.00	−1.20
1 hour	8.00	7.50	−0.50
1 hour	8.80	8.70	−0.10
2 hours	9.85	10.05	+0.20
2 hours	8.80	9.00	+0.20
2 hours	10.10	9.95	−0.15
2 hours	11.30	11.00	−0.30
2 hours	7.10	7.40	+0.30
2 hours	8.60	7.60	−1.00
4 hours	9.25	9.30	+0.05
4 hours	7.40	7.10	−0.30
4 hours	5.15	6.60	+1.45
4 hours	8.80	8.50	−0.30
4 hours	7.10	6.60	−0.50
4 hours	8.65	7.60	−1.05

portions by the Kjeldahl method. The results are given in table VI. The differences in direct reducing sugars, "glucose," are comparatively slight. Those in reducing sugars formed on hydrolysis, "sucrose," are considerably greater, especially when figured as percentages of the total. It is to be remembered, however, that the total amount of sucrose is relatively small, and that the errors in both determinations may accumulate in that of sucrose. It seems

to be impossible to correlate the differences found with the process of bending. The same may be said of the distribution of nitrogen.

Summary

Definite moisture changes accompany geotropic bending in corn nodes. During the early stages of bending there is a greater percentage of moisture in the concave flank. When the process has developed the percentage of water is greater in the convex flank.

Although titration acidity is greater in the convex flank, the differences are very slight. The results on hydrogen ion concentration, although uniform in direction, are not numerous enough to serve as a basis for conclusions.

It is impossible, with the data obtained, to correlate the geotropic bending of etiolated *Vicia Faba* shoots with differences in

TABLE VI

ANALYSES OF *Vicia Faba* SHOOTS EXPOSED TO GRAVITY (IN PERCENTAGE OF FRESH WEIGHT)

Time of Exposure	Upper flank	Lower flank	Difference	Upper flank	Lower flank	Difference
	Glucose			Sucrose		
30 minutes...	2.16	2.15	−0.01	0.450	0.279	−0.171
1 hour.....	1.47	1.40	−0.07	0.187	0.287	+0.100
2 hours.....	1.56	1.67	+0.11	0.221	0.269	+0.048
4 hours.....	1.37	1.41	+0.04	0.449	0.289	−0.160
	Total sugars			Moisture		
30 minutes...	2.61	2.43	−0.18	91.57	92.39	+0.82
1 hour.....	1.65	1.69	+0.04	92.54	92.46	−0.08
2 hours.....	1.78	1.94	+0.16	91.62	91.82	+0.20
4 hours.....	1.82	1.70	−0.12	91.93	92.13	+0.20
	Soluble nitrogen			Insoluble nitrogen		
30 minutes...	0.294	0.301	+0.007	0.302	0.284	−0.018
1 hour.....	0.261	0.259	−0.002	0.303	0.306	+0.003
2 hours.....	0.279	0.305	+0.026	0.314	0.296	−0.018
4 hours.....	0.264	0.258	−0.006	0.349	0.324	−0.025

moisture, titration acidity, hydrogen ion concentration, catalase activity, or the distribution of sugars and nitrogen containing substances.

The writer wishes to express his thanks to Dr. WM. CROCKER for his continued interest in the work, and for his many helpful suggestions.

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EFFECT OF SALTS UPON OXIDASE ACTIVITY OF APPLE BARK

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(WITH FIVE FIGURES)

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(WITH FIVE FIGURES)

Introduction

In an earlier paper (21) one of the authors showed that there is a marked difference in the action of the salts of the alkali metals upon the fire-holding capacity of tobacco, even when the salts have similar anions. For instance, the carbonates of potassium, rubidium, and caesium promote the combustion of tobacco to a very much greater extent than the carbonates of sodium and lithium. The chlorides of sodium, lithium, and potassium retard the combustion, but the chloride of potassium is not nearly so effective as the chloride of sodium or lithium. In general, the salts of potassium, rubidium, and caesium are much more favorable to combustion than those of sodium and lithium.

It has been known for a long time that potassium is an essential element for the higher plants. Numerous attempts have been made to replace potassium by sodium, and, while apparently sodium can fulfil some of the functions of potassium, attempts to replace potassium entirely by sodium have been unsuccessful. The fact that potassium seems to have such a marked property of promoting the combustion of tobacco, and sodium does not, suggests that this particular property of potassium may have a relation to certain functions in the plant, which cannot be fulfilled by sodium. These facts suggested that a study of the effect of the alkali salts upon oxidase activity might be of interest. The work reported in this paper was done in 1917. More extended studies were planned, but, since it has been impossible to carry them out completely at the present time, it seemed wise to report the results obtained.

Historical

BERTRAND (5) was the first investigator to point out that the salts of metals influence oxidase activity. He showed that manganese salts greatly increase the oxidase activity of preparations from alfalfa. GESSARD (15) found that the formation of melanin from tyrosin is increased in the presence of salts of the metals. BACH (4) substantiated GESSARD'S results, and showed that aluminum sulphate, salts of calcium, magnesium, manganese, and zinc increase melanin formation from tyrosin. The effect of the salts is to increase the further change of the oxidation product rather than to activate the taking up of oxygen. Aluminum salts hasten the formation of purpurogallin from the yellow oxidation product of the action of oxidase upon pyrogallol. BACH believed that the oxidation process is retarded by the accumulation of the primary oxidation products, and that the salts act to release them. WOLFF (32) found that the oxidation of tyrosin by tyrosinase from *Russula delica* is increased by the addition of small quantities of disodiumphosphate. PORODKO (26), ASO (3), ALSBERG (2), and EWART (11) have shown that salts of the metals give a blue color with guaiacum. PORODKO and EWART believed these salts to be inorganic oxidases. PORODKO pointed out that those metals which form salts of two degrees of oxidation are particularly active. ALSBERG, and also EWART, confirmed PORODKO'S observation and found that the chlorides of many of the metals give a blue color with guaiacum. ALSBERG attributed an important part in the reaction to the chlorine. EWART further found that the chlorides, nitrates, and sulphates of the same metal are not necessarily equally powerful in their action. Apparently the chlorides are more active than the sulphates. Various salts were found to act as sensitizers or retardants to oxidase activity. Potassium chloride, potassium iodide, potassium bromide, and potassium fluoride retard or even prevent the browning of pounded apple pulp.

Numerous investigators have shown that oxidase activity is affected by changes in reaction of the medium. BERTRAND (6) showed that the action upon guaiacol of laccase from *Rhus succedanea* is inhibited by 0.002 M concentration of sulphuric acid.

Wolff found tyrosinase from *Russula delica* most active in a solution neutral to phenolphthalein, and Abderhalden and Guggenheim (1) found that tyrosinase is destroyed by 0.016 N hydrochloric acid, and greatly retarded by 0.016 N sodium hydroxide. Rose (28) showed that the decrease in oxidase activity, as observed in the Bunzell apparatus, is due to an increase in the hydrogen ion concentration of the medium. Reed (27) found oxidase activity in potatoes and apples inhibited even by low hydrogen ion concentrations; and likewise Bunzell (9) found the action of oxidase retarded with increasing hydrogen ion concentrations.

Methods

All but one of the experiments described in this paper were made with portions of apple bark which had been dried at 35–40° C. for 2–3 hours, ground fine enough to pass through a 40-mesh wire sieve, and stored air dry in zinc-capped Mason jars. One experiment was made with solutions of precipitated oxidase separated from aqueous extracts of healthy bark and of diseased bark by the addition of about 10 volumes of alcohol. In order to obtain the precipitated oxidase, 2 gm. of bark were allowed to stand in a beaker with 10 cc. of water and 5 drops of toluol for 1 hour. The extract was then squeezed out through moist cheesecloth on coarse filter paper. The beaker was washed with five 1 cc. portions of water and the filter paper finally with two more. There was then added 50 cc. of 95 per cent alcohol to the filtrate (concentration of alcohol about 70 per cent) and the whole allowed to stand for 10 minutes. The flocculent precipitate which had formed was collected on a hard filter by gentle suction with a filter pump. There was then added 150 cc. more alcohol to the filtrate (concentration of alcohol now about 90 per cent) and the whole allowed to stand for 1 hour, since precipitation was slow, before this second fraction was collected on the filter with the first. The precipitate was dissolved in water and used immediately, as described later.

The stock solutions of all of the salts tested were made to a concentration of 0.5 N. Potassium chloride, manganese chloride, ferrous chloride, and ferric chloride were used also in the additional

concentrations of 0.1 N and 0.01 N. Since there was always 5 cc. of water in the apparatus, the final concentration of the salt, there was 0.1 N for 0.5 N solutions and 0.02 and 0.002 N for 0.1 N and 0.01 N solutions used.

Oxidation was measured in centimeters of mercury rise by means of the simplified BUNZELL apparatus (8). The shaking machine was run at the rate of 106 complete excursions per minute. All experiments were run for 3 hours, readings being taken every 15 minutes, and a final reading the following morning. When bark was used, the mixtures in the apparatus contained 0.1 gm. of bark, 1 cc. of salt solution, and 4 cc. of 1 per cent pyrogallol solution or salt and pyrogallol with bark omitted, the second combination serving as a control on the first. Preliminary experiments had shown that during the time in which these experiments were run the autoxidation of the pyrogallol was usually not more than the equivalent of 0.15 cm. mercury rise. In the experiment with precipitated oxidase, the precipitate from 2 gm. of bark was dissolved in 20 cc. of water, and 2 cc. of the solution, containing the dissolved precipitate obtained from 0.2 gm. of bark, were put in each apparatus, together with the usual amount of pyrogallol and water. All tests were run in duplicate. Two controls were run with each experiment, one containing only water, the other bark (or oxidase solution), pyrogallol, and water, but without the addition of salts.

The figures for P_H given in table VII were obtained by means of the apparatus described by ROSE (28).

Discussion

The chlorides in general retard oxidase activity. The chlorides of potassium, sodium, and lithium depress markedly the oxidation of pyrogallol by bark (table I). Similar results were obtained with all the other chlorides tested, except ferrous chloride (table VI). Ferrous chloride in 0.1 N concentration with bark and pyrogallol showed 1.79 cm. mercury rise, and with pyrogallol alone 1.45 cm., compared with the control of pyrogallol and bark as 1.00 cm. Since ferrous chloride is readily oxidized when exposed to the air, it is quite probable that the oxygen absorption for the most part represents that absorbed in the oxidation of ferrous chloride.

Results

The results of the experiments are shown in tables I–VII and figs. 1–5.

TABLE I

EFFECT OF 0.1 N KCl, NaCl, AND LiCl ON OXIDATION OF PYROGALLOL BY POWDERED HEALTHY APPLE BARK; TEMPERATURE 23.2–23.6° C.*

TIME OF READING	NO BARK			BARK			
	KCl	NaCl	LiCl	Check	KCl	NaCl	LiCl
May 21							
12.30.....	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.45.....	0.03	0.13	0.00	0.03	0.06	0.07	0.03
1.00.....	0.03	0.13	0.00	0.08	0.05	0.11	0.05
1.15.....	0.08	0.20	0.00	0.23	0.15	0.18	0.15
1.30.....	0.05	0.17	0.02	0.25	0.15	0.18	0.15
1.45.....	0.05	0.13	0.00	0.33	0.15	0.21	0.15
2.00.....	0.07	0.18	0.00	0.38	0.15	0.24	0.16
2.15.....	0.08	0.19	0.05	0.43	0.19	0.27	0.21
2.30.....	0.08	0.19	0.04	0.45	0.19	0.31	0.25
2.45.....	0.07	0.17	0.05	0.45	0.25	0.30	0.25
3.00.....	0.05	0.16	0.05	0.50	0.23	0.32	0.26
3.15.....	0.09	0.19	0.06	0.65	0.26	0.36	0.29
3.30.....	0.10	0.20	0.05	0.68	0.28	0.35	0.32
May 22							
8.40.....	0.00	0.00	0.00	1.25	0.80	0.74	0.77

* In tables I–V manometer readings in cm. of mercury corrected against an apparatus containing only water.

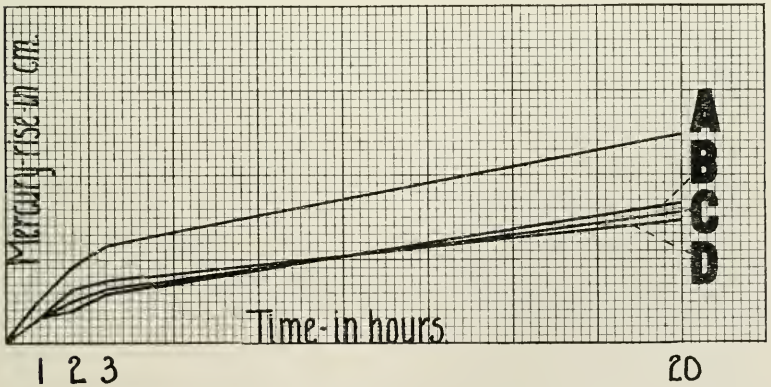

FIG. 1.—Effect of KCl, NaCl, and LiCl on oxidation of pyrogallol by powdered healthy apple bark: A, control (bark and pyrogallol); B, KCl+bark and pyrogallol; C, NaCl+bark and pyrogallol; D, LiCl+bark and pyrogallol.

TABLE II

EFFECT OF 0.10 N ALKALI CARBONATES ON OXIDATION OF PYROGALLOL BY POWDERED HEALTHY APPLE BARK; TEMPERATURE 29.3–30.0° C.

TIME OF READING	NO BARK			BARK			
	K_2CO_3	Na_2CO_3	Li_2CO_3	Check	K_2CO_3	Na_2CO_3	Li_2CO_3
June 13							
1.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.45	0.55	0.50	0.54	0.14	0.08	0.10	0.13
2.00	1.35	1.13	1.19	0.19	0.48	0.47	0.48
2.15	1.75	1.55	1.64	0.24	0.75	0.85	0.82
2.30	2.10	1.78	1.92	0.34	0.93	1.02	1.02
2.45	2.40	2.05	2.12	0.44	1.16	1.27	1.23
3.00	2.50	2.15	2.24	0.49	1.33	1.42	1.33
3.15	2.60	2.28	2.34	0.54	1.44	1.52	1.50
3.30	2.90	2.41	2.55	0.63	1.63	1.76	1.74
3.45	3.00	2.47	2.57	0.68	1.79	1.86	1.85
4.00	3.05	2.54	2.64	0.74	1.87	1.97	1.93
4.15	3.08	2.60	2.69	0.79	1.93	2.02	1.99
4.30	3.17	2.68	2.75	0.86	2.08	2.19	2.22
June 14							
8.30	3.17	2.63	2.74	1.29	2.70	2.61	2.85

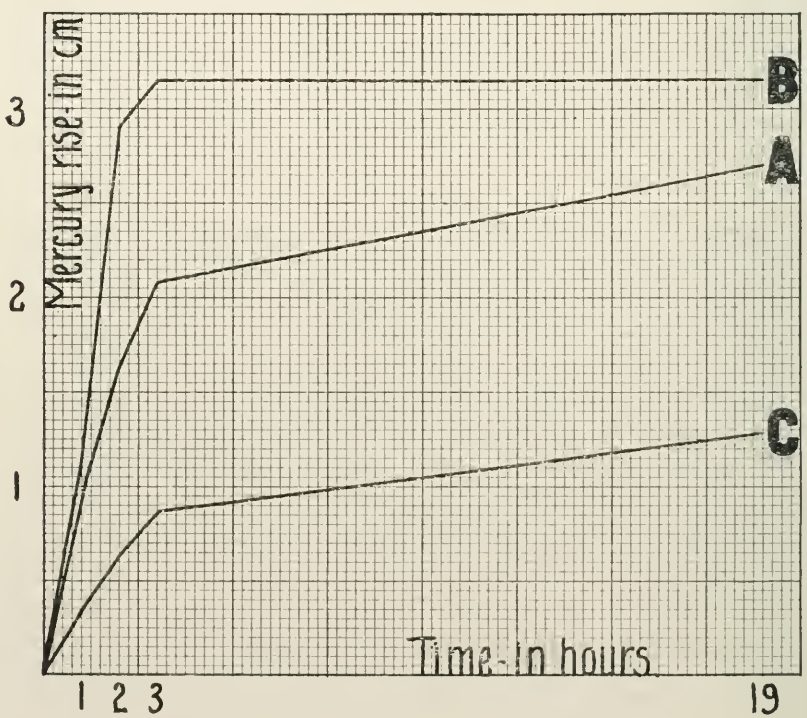

FIG. 2.—Effect of K_2CO_3 on oxidation of pyrogallol, with and without bark (healthy): A, K_2CO_3+bark and pyrogallol; B, K_2CO_3+pyrogallol; C, control (bark and pyrogallol).

TABLE III

EFFECT OF 0.10 N KCl AND K_2CO_3 ON OXIDATION OF PYROGALLOL BY POWDERED DISEASED APPLE BARK; TEMPERATURE 27.8–29.0° C.

TIME OF READING	NO BARK		BARK		
	K_2CO_3	KCl	Check	K_2CO_3	KCl
March 10					
10.00	0.00	0.00	0.00	0.00	0.00
10.15	0.68	−0.05	0.13	0.46	0.16
10.30	1.24	0.00	0.30	0.90	0.33
10.45	1.65	0.00	0.50	1.25	0.38
11.00	1.98	−0.08	0.65	1.50	0.48
11.15	2.25	−0.03	0.72	1.72	0.60
11.30	2.38	−0.03	0.85	1.93	0.69
11.45	2.52	0.00	0.99	2.09	0.82
12.00	2.65	−0.05	1.04	2.22	0.88
12.15	2.75	0.00	1.15	2.35	0.95
12.30	2.78	−0.05	1.18	2.39	0.95
12.45	2.85	−0.08	1.25	2.55	1.00
1.00	2.99	−0.05	1.38	2.65	1.10
March 11					
9.45	3.53	−0.10	2.20	3.73	1.73

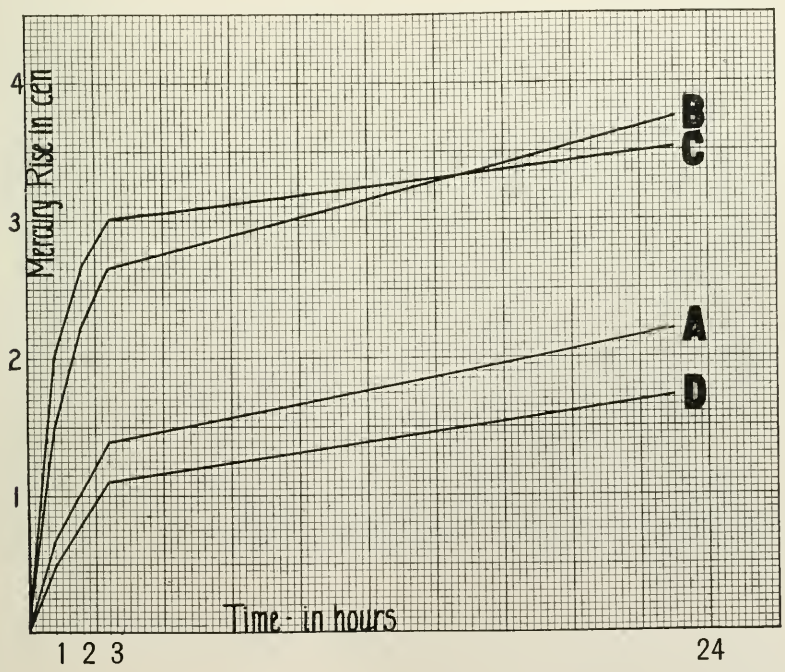

FIG. 3.—Effect of KCl and K_2CO_3 on oxidation of pyrogallol with and without bark (diseased): A, control (bark and pyrogallol); B, K_2CO_3+bark and pyrogallol; C, K_2CO_3+pyrogallol; D, KCl+bark and pyrogallol (KCl+pyrogallol gave no oxidation).

TABLE IV

EFFECT OF 0.10 N POTASSIUM TARTRATE, SODIUM OXALATE, AND $Ca(NO_3)_2$ ON OXIDATION OF PYROGALLOL BY POWDERED HEALTHY APPLE BARK; TEMPERATURE 29.2–30.2° C.

TIME OF READING	NO BARK			BARK			
	Potassium tartrate	Sodium oxalate	$Ca(NO_3)_2$	Check	Potassium tartrate	Sodium oxalate	$Ca(NO_3)_2$
June 22							
1.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.45	0.11	0.08	0.18	0.10	0.14	0.20	0.08
2.00	.....	.....	.....	0.25	0.28	0.25	0.20
2.15	0.25	0.10	0.20	0.30	0.36	0.35	0.23
2.30	.....	.....	.....	0.38	0.48	0.46	0.30
2.45	0.35	0.19	0.20	0.43	0.58	0.55	0.35
3.00	.....	.....	.....	0.55	0.64	0.64	0.40
3.15	0.48	0.31	0.28	0.58	0.76	0.78	0.50
3.30	.....	.....	.....	0.70	0.98	0.80	0.55
3.45	.....	.....	.....	0.73	0.95	0.96	0.59
4.00	0.68	0.38	0.35	0.80	1.03	1.00	0.60
4.15	.....	.....	.....	0.90	1.13	1.10	0.73
4.30	0.78	0.35	0.38	0.90	1.15	1.13	0.70
June 23							
8.20	0.95	0.68	0.23	1.20	1.53	1.60	0.98

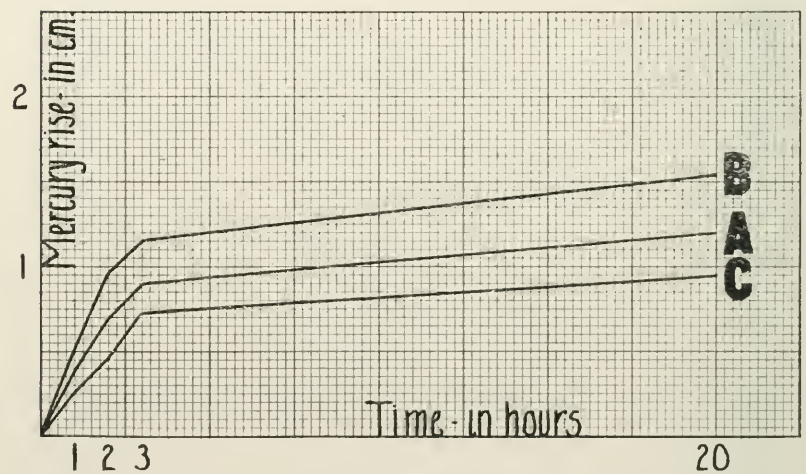

FIG. 4.—Effect of potassium tartrate on oxidation of pyrogallol with and without bark (healthy): A, control (bark and pyrogallol); B, potassium tartrate+bark and pyrogallol; C, potassium tartrate+pyrogallol.

TABLE V

EFFECT OF 0.10 N $MnCl_2$ AND K_2SO_4 ON OXIDATION OF PYROGALLOL BY PRECIPITATED OXIDASE FROM BOTH HEALTHY AND DISEASED APPLE BARK; TEMPERATURE 29.5–30.2° C.

TIME OF READING	HEALTHY			DISEASED		
	Check	$MnCl_2$	K_2SO_4	Check	$MnCl_2$	K_2SO_4
June 21						
1.45	0.00	0.00	0.00	0.00	0.00	0.00
2.00	0.07	0.08	0.11	0.17	0.15	0.15
2.15	0.08	0.10	0.21	0.37	0.29	0.30
2.30	0.08	0.13	0.23	0.42	0.21	0.33
2.45	0.08	0.13	0.27	0.48	0.25	0.43
3.00	0.08	0.10	0.25	0.50	0.23	0.48
3.15	0.15	0.11	0.28	0.56	0.24	0.54
3.30	0.15	0.08	0.30	0.65	0.26	0.58
3.45	0.18	0.09	0.35	0.70	0.29	0.63
4.00	0.20	0.08	0.34	0.79	0.31	0.69
4.15	0.20	0.08	0.37	0.87	0.34	0.78
4.30	0.23	0.09	0.38	0.88	0.35	0.78
4.45	0.28	0.18	0.43	0.98	0.40	0.93
June 22						
8.00	0.53	0.28	0.58	1.24	0.63	1.28

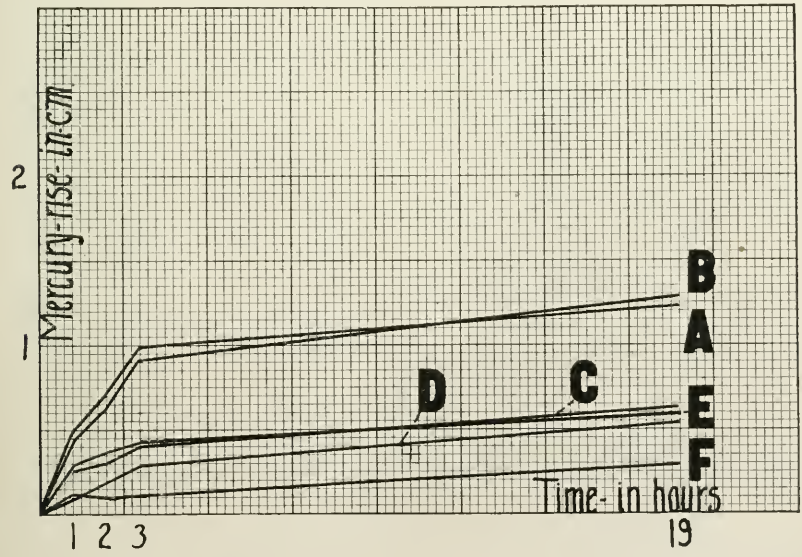

FIG. 5.—Effect of $MnCl_2$ and K_2SO_4 on the oxidation of pyrogallol on precipitated oxidase from both healthy and diseased bark: A (diseased), K_2SO_4+bark and pyrogallol; B (diseased), control (bark and pyrogallol); C (diseased), $MnCl_2$+bark and pyrogallol; D (healthy), K_2SO_4+bark and pyrogallol; E (healthy), control (bark and pyrogallol); F (healthy), $MCnl_2$+bark and pyrogallol.

TABLE VI

SUMMARY OF EFFECT OF ALL SALTS TESTED ON OXIDATION OF PYROGALLOL WITH AND WITHOUT BARK; ALL FIGURES CALCULATED TO BASIS OF 1 CM. MERCURY RISE FOR CHECK.

Salt	Check	Cl		SO_4		NO_3		CO_3		PO_4		H_2PO_4		Tartrate		Oxalate		Acetate		Citrate	
		With	Without	With	Without	With	Without	With	Without	With	Without	With	Without	With	Without	With	Without	With	Without	With	Without
K 0.1 N...	1.00	0.63	0.00	1.07	0.08	0.99	0.07	2.09	2.46	1.85	1.90	0.96	0.16	1.27	0.76	1.16	.....	.....	.....	.....	.....
K 0.02 N..	1.00	1.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.04	.....	.....	.....	.....	.....	.....	.....	.....	.....
K 0.002 N.	1.00	1.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.07	.....	.....	.....	.....	.....	.....	.....	.....	.....
Na 0.1 N...	1.00	0.59	0.00	1.06	0.06	0.98	0.09	2.02	2.11	.....	.....	.....	.....	1.14	0.26	1.33	0.55	1.55	1.84	2.06	2.41
Li 0.1 N...	1.00	0.61	0.00	1.05	0.10	....	.....	2.20	2.19	.....	.....	.....	.....	.....	.....	.....	.....	1.37	1.28	1.92	2.09
Cs 0.1 N...	1.00	0.66	0.10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
NH_4 0.1 N...	1.00	0.69	0.17	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Ca 0.1 N...	1.00	0.57	0.15	.....	.....	0.80	0.15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mg 0.1 N...	1.00	.....	.....	.....	.....	0.94	0.12	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mn 0.1 N...	1.00	0.54	0.10	1.04	0.02	0.76	0.04	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mn 0.02 N..	1.00	1.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mn 0.002 N.	1.00	1.15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Ba 0.1 N...	1.00	.....	.....	.....	.....	0.84	0.10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fe''' 0.1 N...	1.00	1.79	1.45	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fe''' 0.02 N..	1.00	1.12	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fe''' 0.002 N.	1.00	1.10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fe'' 0.1 N...	1.00	0.24	0.05	.....	.....	0.31	0.24	.....	.....	.....	.....	.....	.....	.....	.....	0.45	0.06	.....	.....	.....	.....
Fe'' 0.02 N..	1.00	0.55	.....	.....	.....	0.55	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fe'' 0.002 N.	1.00	0.86	.....	.....	.....	1.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mn 0.1 N...	1.00	0.50	.....	1.03	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
K 0.1 N...	1.00	0.52	.....	1.09	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

TABLE VII

RELATION OF OXIDATION TO INITIAL PH OF MIXTURES*

SALT	CHECK		Cl		SO_4		NO_3		CO_3		H_2PO_4		TARTRATE		OXALATE		ACETATE		CITRATE	
	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H	Oxidation	P_H
K	1.00	5.15	0.63	5.19	1.07	5.13	0.99	5.14	....	.....	0.96	4.47	1.27	6.00	1.16	5.77	.....	5.72	.....	5.65
Na	.....	.....	0.59	5.17	.....	.....	.....	.....	.....	.....	.....	.....	1.14	.....	1.33	.....	1.55	6.43	2.06	6.02
Li	.....	.....	.....	.....	1.05	5.06	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.37	5.97	1.92	6.19
NH_4	.....	.....	0.69	4.85	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Ca	.....	.....	0.57	4.79	.....	.....	0.80	4.74	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mg	.....	.....	.....	.....	.....	.....	0.94	4.62	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mn	.....	.....	0.54	4.48	1.04	4.50	0.76	4.43	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Ba	.....	.....	.....	.....	.....	.....	0.84	4.78	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fe	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fe''	.....	.....	0.24	1.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

* Concentration of salts 0.10 N.

This view is substantiated by the fact that when the concentration of ferrous chloride is reduced, oxygen absorption is reduced proportionally (table VI). If we subtract 1.45 cm. (mercury rise for pyrogallol and ferrous chloride) from 1.79 cm. (mercury rise for bark, pyrogallol, and ferrous chloride), we have 0.34 cm. for the oxidase activity of the bark in the presence of the ferrous chloride as compared with 1.00 cm. for the oxidase activity of bark and pyrogallol in the absence of ferrous chloride. Apparently ferrous chloride retards oxidase activity just as the other chlorides do, and the increased absorption of oxygen in the presence of ferrous chloride is due to the action of ferrous chloride itself in absorbing oxygen. Oxidation is increased by 0.002 N manganese chloride. This is in accord with the results of BERTRAND (5 and others. In a concentration of 0.1 N it inhibits oxidation just as do the other chlorides.

The use of precipitated oxidase shows that chlorides have a depressing effect on oxidation, even under conditions which eliminate many of the substances present in the bark powder. No investigation has been made of the effect of these substances on the reaction, but they probably complicate it.

The results with the chlorides are in accord with the work of EWART, who found that dilute solutions of potassium chloride and sodium chloride prevent the browning of slices of apples. EWART'S further conclusion, however, that the chlorides act as sensitizers to oxidation, or ALSBERG'S idea that chlorine plays an important part in the bluing of guaiacum by the chlorides of metals, are scarcely borne out by our observations that chlorides in general depress oxidase activity. It should be noted, however, that the results of those investigators were based upon color reactions, while ours were based upon oxygen absorption.

It is interesting to note that the chlorides which retard the combustion of tobacco at high temperatures have a similar effect in depressing oxidase activity. KRAYBILL (21) has suggested that the chlorides may have a negative catalytic action in the case of the combustion of tobacco. It would be interesting to know how the chlorides affect other oxidation processes.

The depressing effect of chlorides on oxidase activity is in contrast with their action on other enzymatic processes. Thus NASSE (25), KÜBEL (22), COLE (10), WOHLGEMUTH (31), LISBONNE (23), HAWKINS (18), and others have found that chlorides increase the diastatic power of various preparations of diastase. NASSE, however, found that under certain conditions sodium chloride retarded diastatic activity, and later HAWKINS showed that sodium chloride and potassium chloride in certain dilute concentrations (M/128–M/512) retard diastatic activity. It would have been better if the effect of the chlorides upon oxidase activity had been determined in a greater number of concentrations, and it will be well in the future to do so in studying this problem. The effect of salts upon lipase activity is also of interest in this connection. LOEVENHART and PEIRCE (24), GERBER (14), TERROINE (30), HAMSIK (16), FALK (12), and others found that the chlorides of various alkalies and alkaline earths retard lipase activity. TERROINE found that the concentration of the salts which he studied determined the nature of their influence. BUCHNER, BUCHNER, and HAHN (7) found that the chlorides of sodium, calcium, barium, and ammonium inhibit the fermentation of cane sugar or glucose in the presence of pressed yeast.

The results presented in table VI do not show any marked difference in the behavior of the different chlorides tested. The cations, judging from the limited data available, apparently have little or no effect; or at least their chlorides all behave very much in the same manner. In this respect the alkali salts are different in their effect upon the fire-holding capacity of tobacco, for here the salts of caesium, rubidium, and potassium in general are much more favorable to combustion than the corresponding salts of sodium or lithium. A similar contrasting behavior of different cations of chlorides was noted by HARDEN (17), who found that potassium chloride and ammonium chloride cause a definite degree of fermentation in inactivated yeast, while sodium chloride has no effect. He says: "A specific difference in relation to alcoholic fermentation exists between the ions of sodium on the one hand and of potassium and ammonium on the other hand." SCHREINER and SULLIVAN (29) found that potassium salts retard oxidation by the roots of plants.

The effect of the chlorides of the alkalies in retarding oxidase activity suggests a possible practical application in preventing the browning of fruits and vegetables during their preparation for canning, preserving, or drying.

The sulphates apparently increase oxidation slightly in all cases, but the readings are not sufficiently large to be of any positive significance.

The nitrates of potassium, sodium, and magnesium have no marked effect on oxidation, while the nitrates of barium, calcium, manganese, and iron (ferric) decrease it. These results are similar to the effect upon respiration as found by ZALESKI and REINHARD (33). FERNBACH and LANZENBERG (13) and KAYSER (20) find that nitrates increase alcoholic fermentation, but, as they point out, the effect may be to increase multiplication of the yeast cells rather than to affect enzymatic action.

In tables II and III and figs. 2 and 3 are shown the oxidation of pyrogallol by bark alone, by bark and carbonate, and by carbonate alone. From these it is seen that in the last two cases oxidation is considerably greater than that by the bark alone. It is also seen that during the first 3 hours oxidation by carbonate is greater than that by carbonate and bark, but that after the experiment has stood overnight oxidation by healthy bark and carbonate approaches that by carbonate alone, and oxidation by diseased bark and carbonate exceeds it.

The most obvious explanation of this fact, although possibly not the true one, is that oxidation by a carbonate is a strictly chemical reaction, catalyzed only by hydroxyl ions, which soon comes to a definite end, while oxidation by carbonate and bark is a reaction catalyzed by both "oxidase" and hydroxyl ions, in which the presence of the hydroxyl ions increases the effectiveness of the "oxidase," which is slow in reaching an end-point.

Table VI shows that tripotassium phosphate increases oxidation of pyrogallol very markedly, both with and without bark. Although no P_H values for this mixture are available, we know the salt is alkaline in reaction, and this effect complicates the matter. With potassium dihydrogen phosphate at 0.10 N concentration a decrease is evident, and at 0.02 N and 0.002 N concentrations a

slight increase in oxidation occurs. The higher hydrogen ion concentration is probably the cause of the slight depression in oxidation of the 0.10 N strength of the salt. The slight increase in oxidation of the lower concentrations suggests that phosphates may increase oxidase activity, but the limited data are inconclusive. It is interesting to note that IWANOFF (19) found that phosphates raise the amount of respiration in living wheat seedlings. ZALESKI and REINHARD (33) found that disodium phosphate increases the output of carbon dioxide from dried ground seeds, and that the monobasic phosphate decreases it because of the acid reaction. These authors also quote from the work of a student, Miss SCHKLOUSKY, who showed that phosphates increase the action of peroxidases, and from work of another student, Miss ROSENBERG, who showed that phosphates stimulate the catalase activity of different seeds.

In the case of salts of organic acids and the carbonates, all more alkaline than any of the inorganic salts (table VI), oxidation is greater at all stages of the experiment when bark is used than when it is not. Examples of this are shown in table IV. The effect of the salt is not merely additive, however, either here or in the case of the carbonates, as is shown by the following:

OXIDATION OF PYROGALLOL BY BARK AND SALT

	Tested separately (cm. of mercury rise)	Tested together (cm. of mercury rise)
K_2CO_3	4.46	2.70
K tartrate	2.15	1.53
Na oxalate	1.88	1.60

Evidently when bark and salt are combined, there is some factor at work which brings about a slower rate of oxidation than might be expected. What this factor may be we have no means of knowing as yet. Possibly it is the partial neutralization of the hydroxyl ions of the salt by the acid of the bark.

The question why salts vary so widely in the effect they have on oxidation is not easily answered. If we consider only the results with 0.1 N solutions, it seems clear, in the case of the carbonates, potassium dihydrogen phosphate, and the salts of organic acids here reported, that increased oxidation in their presence is due to the excess of hydroxyl ions they furnish; that is, by the

reaction (P_H) their solutions establish when mixed with bark and pyrogallol (table VII). The reaction established by the chlorides, however, can hardly be responsible for the decrease in oxidation they bring about, since sulphates, giving about the same reaction, cause a small increase in oxidation. For example, a mixture of potassium chloride, bark, and pyrogallol has a P_H of 5.19 and gives only 63 per cent as much oxidation as the control. A similar mixture containing potassium sulphate has a P_H of 5.13 and gives 7 per cent more oxidation than the control. The corresponding figures for manganese are: manganese chloride mixture, $P_H = 4.50$, oxidation = 104 per cent of the control.

The situation for nitrates shows several irregularities. Potassium nitrate giving a P_H of 5.14 has practically no effect on oxidation. Magnesium nitrate is also without effect, but gives a P_H of 4.62. The nitrates of calcium, barium, and manganese inhibit oxidation, but manganese gives a lower P_H and the other two a higher one than that given by magnesium nitrate.

The results presented justify the conclusion that when 0.1 N solutions of the salts are used, other ions than hydrogen and hydroxyl play an important part in controlling oxidation. When hydrogen or hydroxyl ions are neutralized in making oxidase activity determinations, therefore, it is important to take into consideration the possible effect of the salts formed thereby. This must be considered as merely preliminary to the real investigations of the relation of specific ions to the oxidation processes in plants and animals. The effect of iron and manganese salts has long been known, but more work is necessary, both with these and with the more commonly occurring chlorides, sulphates, and nitrates of other cations.

Summary

1. One-tenth normal solutions of all of the chlorides tested (potassium, sodium, lithium, caesium, ammonium, calcium, manganese, ferric) decreased oxidation of pyrogallol by apple bark powder.

2. Oxidation was increased very slightly by 0.10 N solutions of all the sulphates tested.

3. Potassium, sodium, and magnesium nitrates (0.10 N) had practically no effect on oxidation, while nitrates of calcium, barium, manganese, and iron (ferric) decreased it.

4. Potassium chloride (0.02 N and 0.002 N) had no effect on oxidation, while manganese chloride in these concentrations increased it.

5. Tartrates, oxalates, citrates, acetates, and carbonates increased oxidation. Marked increase in oxidation in these cases seems to be due, in part at least, to the low acidity of the mixtures of bark, pyrogallol, and salt.

6. Marked decrease in oxidation is not necessarily accompanied by high acidity of the mixtures.

7. Ions other than the hydrogen and hydroxyl may be important in regulating oxidase activity.

8. In neutralizing hydrogen or hydroxyl ions, it is important to take into consideration, in the study of oxidase activity, the possible effect of the salts formed thereby.

9. The chlorides which retard the combustion of tobacco at high temperatures also retard the oxidase action at low temperatures.

10. The effect of the alkali chlorides upon oxidase activity suggests a practical application in preventing the browning of fruits and vegetables during their preparation for canning, preserving, or drying.

The authors wish to express their appreciation to Dr. Wm. Crocker for many helpful suggestions.

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LIFE HISTORY OF FOSSOMBRONIA CRISTULA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 264

Arthur W. Haupt

(WITH PLATES XIV–XIX AND ONE FIGURE)

Fossombronia, according to Schiffner (8), comprises 26 species of world wide distribution. The genus belongs to the family Codoniaceae of Cavers (2), which is, next to the Haplomitriaceae, the highest family of the anacrogynous Jungermanniales. *Fossombronia* and its closely related genera *Blasia*, *Noteroclada*, and *Treubia* are thalloid dorsiventral forms which show the beginnings of genuine leaves corresponding to those of the acrogynous Jungermanniales, and represent, with the Haplomitriaceae, possible ancestral forms from which the Acrogynae have been derived.

Fossombronia cristula was discovered and named by Austin (1) in 1868, who found it growing "on damp sand in an unfrequented path" near Batsto, New Jersey. For many years no additional material was collected, nor was it reported as occurring in any other locality in the United States. This no doubt was due to the small size and obscure habitat of the species. In 1915 Evans (3) made a taxonomic study of *F. cristula* and stated that specimens had been collected in Massachusetts, Connecticut, New York, New Jersey, West Virginia, and Indiana. Land found the species in 1914 in Porter County, Indiana, 2–3 miles east of Dune Park, and a preliminary report of its occurrence in this region was published by Hill (5) in 1916 from material furnished him by Land. Hill also found plants growing in Lake County, Indiana, 3 miles east of Tolleston. In his paper the author incorrectly refers to the species as *F. crispula*, which is not the name given it by Austin.

Material

The material used in this study to illustrate the development of the sporophyte was kindly furnished by Dr. Land from his collection of 1914 from the Dune Park region. Additional plants

were obtained by the writer from the same locality in 1917, about a month earlier than Dr. LAND'S material had been collected, and served to illustrate the development of the thallus and the sex organs. The writer found *F. cristula* in this locality growing in cracks on fine, wet deposits of silt on the bottom of an almost extinct lake. HILL notes that "a favorite place of growth in the Tolleston locality was vertical sides of holes left in the mud by the feet of cattle." In the Dune Park region the plants are associated in great abundance with *Drosera longifolia*.

Historical summary

The earliest detailed study of *Fossombronia* is that of LEITGEB (7), who investigated *F. pusilla*, a European species. The author made a very careful study of the origin and insertion of the leaves and the development of the stem axis and mucilage hairs in the region of the growing point of the thallus. The apical cell is dolabrate, cutting off alternately right and left segments only. The plants are mostly monoecious, and on those in which antheridia are in greatest abundance, archegonia also occur to a limited extent. In regard to the order of appearance of the sex organs, the author says: "Aber ich fand häufig Sprosse mit völlig entwickelten Kapseln, welche nach der Spitze hinwieder reichlich Antheridien producierten." The position of the antheridia and archegonia is the same as that of the other species, and both originate close to the apical cell. In regard to the development of the antheridia it is stated that they deviate in no way from the normal type, although no figures are shown to illustrate this development. The venter of the archegonium is 2 cells thick before fertilization.

The fertilized egg is elongated in the direction of the archegonium axis, and divides by 2 horizontal walls, forming a tier of 3 superimposed cells, of which the lower forms the foot, the middle cell the seta, and the upper one the capsule. The upper and lower cells divide more actively than the middle one. The differentiation of wall cells and sporogenous tissue in the capsular region occurs early. The mature capsule is 2-layered; the inner wall forms annular thickenings. At the apex the capsule wall is 3-layered.

The author studied the germination of the spores; he notes that a dolabrate apical cell is organized early, but he makes no statement regarding the development of the leaves.

The most complete study of *Fossombronia* since LEITGEB is that by HUMPHREY (6), who investigated *F. longiseta*, a species occurring in California. The thallus reaches a length of 30 mm. and develops genuine leaves like the other species of the genus. The plants revive well after undergoing desiccation, and tuber-like thickenings are formed on the stem in which fungi live. The plants are monoecious, or by exception dioecious. HUMPHREY'S account of the development of the antheridium is most interesting, in that it departs widely from the usual Jungermanniales type.

> The initial cell of the antheridium is somewhat larger than the neighboring vegetative cells, and is readily distinguished from them by its deeper staining qualities. . . . . Just previous to the first division the initial cell becomes considerably elongated, extending a third or more of its total length above the surrounding cells. The first division results from the formation of a horizontal wall which cuts off the stalk from the antheridium itself. Unlike what occurs in the majority of the Jungermanniaceae, the next division, instead of being vertical, is horizontal, thus dividing the antheridium mother cell into two superimposed cells; whereas in *Sphaerocarpus* and *Geothallus* another horizontal wall is formed, thus producing another cell, the two uppermost dividing vertically to form the antheridium, while the basal cell, by a series of transverse walls, forms the foot.
>
> In *Fossombronia* the development thus far agrees exactly with that in *Sphaerocarpus* and *Geothallus*, except that in *Fossombronia* only one horizontal division occurs in the antheridium mother cell, the stalk arising from the basal cell formed by the first horizontal division. This basal cell later divides horizontally, the uppermost segment becoming active in the formation of the stalk, while the lower ordinarily does not divide again. Following the horizontal division of the antheridium mother cell are two vertical divisions forming planes at right angles to each other and dividing the antheridium into octants. The next division results in periclinal walls for each of these octants, and there thus arise eight central cells and eight periclinal ones. . . . .
>
> Judging from the development of the antheridium, *Fossombronia* is more closely related to *Sphaerocarpus* and *Geothallus* than to the higher forms of the Jungermanniaceae. . . . . Thus it seems that *Fossombronia longiseta* forms a connecting link between such forms as *Sphaerocarpus* and *Aneura*.

The development of the archegonium presents no striking difference from the usual situation; 6 neck canal cells are formed

and the venter becomes 2 cells thick only after fertilization. The first division of the fertilized egg is transverse, the upper segment forming the capsule and the lower forming the foot. The second transverse division separates the segment which is to form the capsule from that which is to form the seta. A third transverse division occurs in the uppermost cell, resulting in a tier of 4 superimposed cells. After this 2 vertical walls appear at right angles to each other, followed by periclinal walls in the upper segment. The author states that the capsule wall is normally 2 cells thick, but shows a wall composed of 3 layers of cells in his fig. 61. Both layers of the capsule bear annular thickenings. The mature elaters reach a length of 150–300 μ, and are provided with a double spiral thickening. Dehiscence is by means of four valves.

HUMPHREY'S account of the development of the antheridium is vague, especially because no references to his figures are given in the description. Two interpretations are possible. If the second wall in the antheridium initial is transverse and is followed by vertical divisions in the two uppermost segments, the development is exactly like "what occurs in the majority of the Jungermanniaceae," as his figure representing this stage is the same as my fig. 11, except that the first vertical divisions result in an octant of cells instead of the condition shown in fig. 15. If HUMPHREY speaks of the initial as the dorsal segment resulting from the first transverse division of the true initial, then the third wall in the true initial is transverse instead of vertical, but the situation according to this interpretation would be precisely the same as that in *Sphaerocarpus*.

At any rate, HUMPHREY'S series of stages are not sufficiently close to convince one that the situation in *Fossombronia* is radically different from that characteristic of most of the other Jungermanniales, and inasmuch as no mitotic figures are shown to prove the exact sequence of the first divisions in the initial, except for his figures of cross-sections, it is possible to interpret the development of the antheridium of *F. longiseta* as strictly normal. If HUMPHREY is really familiar with the development of the antheridium in the majority of the Jungermanniales as well as that of *Sphaerocarpus*, and the difficulty in interpreting his account is merely the result

of his obscurity in explaining the situation, the development of the antheridium would be as represented in fig. 1.

Investigation

Thallus

The vegetative body of *Fossombronia cristula* is minute, being only 2–4 mm. in length. It is creeping and semi-prostrate, although

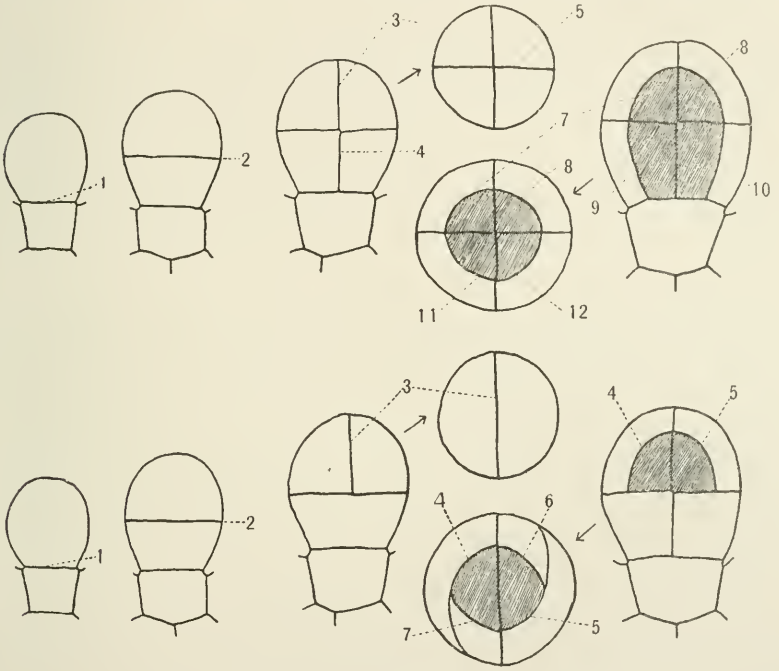

Fig. 1.—Above, *F. longiseta;* below, *F. cristula*

the stem tips may occasionally be more or less ascending. The branching is rather profuse and is strictly apical. The stem shows no indication of a conducting system as in *Pallavicinia* and *Symphyogyna*. The plants form dense matlike growths over the substratum, and are attached by means of long, violet-red rhizoids (fig. 6). The plant is an annual, developing in the early summer as soon as its habitat becomes sufficiently dry; in the Dune Park region the spores are ripe by late September or early October.

Growth of the main axis and branches is by means of a dolabrate (zweischneidig) apical cell (figs. 4, 5), with which are associated simple ventral mucilage hairs (figs. 7, 22) which may be several cells in length. CAVERS (2) states that each lateral segment of the apical cell of *Fossombronia*, by 2 transverse divisions, forms 3 horizontal cells, the upper and lower cells developing the stem and the middle cell forming a leaf, according to the same method as occurs in *Blasia*.

The leaves are borne in 2 dorsal rows; they are more or less erect, obliquely inserted on the stem, closely imbricate, and pale green (fig. 2). The ventral surface of the thallus is entirely devoid of leaves. HILL (5) notes that the leaves become paler and whitish with age. The shape of the leaves varies from somewhat quadrate to slightly obovate; they are very crisped and have subentire margins which occasionally bear a few feeble crenulations at the apex.

The cells of the stem and leaves contain numerous small peripheral chloroplasts. Considering the small size of the plant, the cells are relatively large. Mitotic divisions were very rare in the material studied; the best mitosis seen was that of a late metaphase in the apical cell (fig. 3). From a study of this figure it was estimated that the haploid number of chromosomes is 4, although this fact cannot be stated with absolute certainty, as no other stages of mitosis equally favorable for chromosome counting were found.

There can be no doubt that the 2 rows of lateral outgrowths from the axis of *Fossombronia* represent true leaves. The development of such a plant body from a form like *Pallavicinia Lyellii*, which consists of a midrib with thin, one-layered lateral wings slightly undulate on the margins, is very logical. *Symphyogyna aspera* might be taken to illustrate a second evolutionary stage, as in this plant the wing margins of the thallus are distinctly lobed. Among the Codoniaceae, *Blasia* represents a still farther advance, as in this case the lobes are even more distinct and regular, and the step from this condition to that of *Fossombronia* is perfectly natural. The plant body of *Noteroclada* is still more distinctly leafy, and in *Treubia* the axis bears 3 rows of leaves formed by an

apical cell of the pyramidal type. This series, of course, is not a truly phylogenetic one, but represents a sequence of hypothetical stages through which the Jungermanniaceae acrogynae have probably passed in the course of their evolution.

Sex organs

The plants of *Fossombronia cristula* are monoecious; the sex organs are dorsal and scattered over the stem in the leaf axes. The antheridia and archegonia are more or less separately grouped, but both kinds may occur in the same leaf axis (figs. 7, 8). There is no time relation in the appearance of the sex organs; antheridia may precede or follow the archegonia, and this sequence may be repeated several times in any order.

The question of the differentiation of sex in *F. cristula* is an interesting one. Inasmuch as the thallus is bisexual and there is no definite sequence of antheridia and archegonia, sex must be determined at some other point in the life history than at the reduction division, or at one of the divisions of the apical cell. Up to the formation of the first horizontal wall in the initial, no differentiation of sex has occurred. Moreover, as the first vertical wall determines the kind of sex organ to be produced, sex probably is determined at the division concerned with the formation of the first gametogenous cell. It would be an interesting experiment to attempt to control sex in this plant by external conditions, as the sex organ initials probably contain the possibilities of both sexes.

Antheridium.—The antheridia develop in small groups, either separately or with archegonia, in acropetal succession from the immediate dorsal segments of the apical cell. Each group comes to lie in the axis of a leaf which acts as an involucral organ, protecting the group from behind. There is no special involucre developed, as in many of the strictly thallose Jungermanniales, for, as the writer has pointed out in his study of *Pallavicinia* (4), the antheridial involucre of the thallose forms is strictly homologous with the involucral leaf of the foliose forms.

In the development of the antheridium of *F. cristula*, the initial becomes papillate (fig. 9), and by a transverse division a basal cell is cut off from an outer cell. A second transverse wall

then divides the outer cell into equal segments, forming a primary stalk cell and a primary antheridial cell (fig. 10). The next division is vertical in the antheridial cell, and is usually followed by a similar division in the stalk cell (fig. 11), which may be parallel with or at right angles to the vertical wall in the antheridial cell (figs. 13, 14). Two periclinal walls then appear in the antheridial cell (figs. 13, 14); their relation to the first vertical wall may best be seen in a cross-sectional view (fig. 15). Two additional periclinal walls, which come in at right angles to the first two, complete the peripheral layer of 4 primary wall cells, which are thus separated from the 2 central spermatogenous cells (fig. 15). The cell contents of the primary spermatogenous cells assume a much darker stain than the contents of the primary wall cells or the cells of the stalk; in no cases were periclinal walls seen in the stalk cell. Thus there can be no doubt that the antheridium develops according to the usual method found among the anacrogynous Jungermanniales, and not as HUMPHREY has described for *F. longiseta*.

Occasionally a transverse wall may appear in the stalk cell before the periclinal walls are formed in the antheridial cell (fig. 12), but usually the divisions of the stalk cell follow the formation of the primary wall cells. Sometimes, also, the first division of the stalk cell may be transverse instead of vertical (fig. 16). Further development of the spermatogenous tissue is like that of the other Jungermanniaceae anacrogynae. The stalk of the mature antheridium is commonly 4 cells in length, and invariably shows 4 cells in cross-section. The sperms are very small, slender, and extremely coiled before their escape from the antheridium. Each bears a pair of long terminal cilia. The sperms are produced in pairs from the sperm mother cells, but their development is not favorable for critical cytological study because of their extremely small size.

ARCHEGONIUM.—The archegonium originates from a papillate initial which may be formed from the first segment of the apical cell (figs. 21–23). This feature brings *Fossombronia* very close to the acrogynous Jungermanniales. In no case was an archegonium seen arising directly from the apical cell; consequently its activities are not checked by the production of sex organs.

The first wall of the initial is transverse, and comes in above the general level of the thallus, resulting in the formation of a basal cell and an outer cell (figs. 22–24). The former may undergo another transverse division immediately, or it may remain undivided until the 3 vertical walls have appeared in the outer cell (fig. 26). The presence of 2 transverse walls in the young archegonium caused the writer, during the early part of the investigation, to suspect that possibly the first transverse division of the initial is followed by a second one in the outer cell before the coming in of the 3 vertical walls. Archegonia were seen, however, in which only one transverse division of the initial had taken place (fig. 25), and the indications were that the development of the archegonium may be typical, or that the first 2 divisions of the archegonium initial may be the same as the first 2 of the antheridium initial (fig. 10).

Before the appearance of the first vertical wall, archegonia cannot be distinguished from antheridia, and after the first vertical wall has appeared the mitotic figure which would settle this point has disappeared. In several cases, however, the wall in the basal cell had not become thickened. This fact, together with the general aspect and behavior of the neighboring cells of the thallus, the position of the first wall in the initial, and the elongated character of the undivided stalk cell, convinced the writer, after a study of all available stages in the preparations, that the second transverse wall comes in the basal cell and not in the outer cell.

Subsequent development of the archegonium agrees with the usual development of the archegonium of anacrogynous forms (figs. 27–31). The cover cell divides by a median vertical wall soon after its formation (fig. 29), and remains in this condition; thus it does not contribute to the development of the neck, the cells of which in all cases increase by intercalary divisions. The mature archegonium has 6–8 neck canal cells, surrounded by 5 rows of neck cells (fig. 32). The venter is 2 cells in thickness, and slender, and the neck but slightly twisted. The ventral canal cell and egg are almost equal in size (fig. 31). After the breaking down of the axial row the protoplast of the egg is withdrawn somewhat from its wall, the very dense chromatin is in close contact with the nucleolus, and elongated slender plastids

are conspicuous in the cytoplasm (fig. 33). The egg protoplast does not lay down a new wall until after fertilization. More than one archegonium in a group may function (fig. 45).

That the archegonium is of an advanced type is shown by its early development from the initial, its relatively few neck canal cells, its inactive cover cell, the intercalary growth of the neck, and its slender venter.

Sporophyte

The first division of the fertilized egg is invariably transverse, and is followed by transverse divisions up to 5–7, the sequence of which could not be determined (figs. 34–36). A vertical wall then appears, intersecting the transverse walls (fig. 37), and followed by another vertical wall at right angles to the first one, so that 4 cells are seen in cross-section. Periclinal walls then appear in the upper part of the embryo and a sterile wall is thereby cut off from the central primary sporogenous cells. The relation of the early divisions of the embryo to the formation of the foot, seta, and capsule could not be determined, but it is certain that the lower half of the fertilized egg contributes to the development of the sporophyte, not merely forming an appendage to the foot. A slender calyptra 3 or 4 cells in thickness is formed from the venter of the archegonium (figs. 35, 38). A simple, bell-shaped involucre develops after fertilization; it slightly exceeds the sporophyte in length (fig. 45).

The sporogenous tissue is differentiated early in the history of the sporophyte. In the formation of the spore mother cells and elaters, the protoplasts of the sporogenous tissue withdraw from their cell walls (fig. 39), those which are to form spores round out, and both the spore mother cells and young elaters form a new wall as the original walls of the sporogenous mass are dissolved (fig. 40). The spore mother cells and young elaters are derived from the sporogenous cells by the same number of cell divisions. In *F. cristula* an elater is not homologous with a row of spore mother cells, as in forms with a more highly specialized sporophyte, but with a single spore mother cell. The spore mother cells develop 4 inconspicuous lobes (fig. 42), the reduction divisions

occur, and walls come in to separate the 4 members of the tetrad (fig. 43).

The material available for the investigation yielded no stage beyond that shown by fig. 44. No spiral thickenings were visible on the wall of the elaters, and the spores were in various stages of separation from their tetrads. The seta at this stage is not yet elongated. EVANS (3) has made a careful study of the mature spores and elaters of this species. He says:

> The elaters . . . . are remarkable not only on account of their small size and delicate structure but also on account of their variability in form and scanty development. Their most usual features, however, are found in the local thickenings on their walls. Instead of forming 2 or more parallel spirals, these usually consist of from 5 to 9 rings, some of which may be connected to form a single rudimentary spiral. . . . . The elaters vary from 28 μ to 50 μ in length and from 6 μ to 18 μ in width. The bands of thickening are less deeply pigmented than in most species of *Fossombronia* and are sometimes very pale indeed and difficult to demonstrate. . . . . The brown spores in the type material are mostly between 36 μ and 40 μ in diameter. . . . . The spherical face is covered over with a more or less regular reticulum formed by intersecting lamellae about 2 μ in height. . . . . The meshes of the reticulum are mostly 8–10 μ wide and the spherical face usually measures 6 or 7 meshes across. Sometimes the reticulum is irregular or incomplete.

The mature capsule is globular or nearly so; its wall is invariably 2 cells thick and bears rudimentary annular and half-ring fibers on the walls of the inner layer (fig. 46). There is no sterile cap at the apex of the capsule. Dehiscence, according to CAVERS (2), is by means of 4 valves in some species of *Fossombronia*, but in most of them the upper part of the capsule breaks into plates which are cast off irregularly.

Summary

1. The vegetative body of *F. cristula* consists of a minute, creeping, rather profusely branched thallus which bears genuine leaves in 2 dorsal rows.

2. The apical cell is dolabrate. Branching is strictly apical.

3. The plants are monoecious, the sex organs occurring in the axes of the leaves. Antheridia and archegonia may occur in the same leaf axis, and there is no time relation in the order of their

appearance. They originate from the immediate segments of the apical cell, and their development is strictly acropetal.

4. The antheridia develop according to the usual method found among the anacrogynous Jungermanniales. Variations occur in the order of appearance of the walls in the primary stalk cell.

5. Until the appearance of the first vertical wall, young archegonia cannot be distinguished from young antheridia. The first transverse division in the archegonium initial separates the stalk cell from the archegonium proper, and subsequent development follows the usual Jungermanniales type. The cover cell is inactive, 6–8 neck canal cells are formed, and the venter is 2 cells thick before fertilization. The archegonium is of an advanced type.

6. The early divisions of the embryo are transverse, both halves of the fertilized egg contributing to the development of the foot, seta, and capsule. A calyptra 3–4 cells in thickness is formed.

7. The sporogenous tissue is differentiated rather early in the history of the sporophyte. The elaters are rudimentary, and each is homologous with a single spore mother cell, not with a row of them.

8. The sporophyte is primitive.

To Dr. W. J. G. LAND, under whose direction the study was made, the writer makes grateful acknowledgment for his kind advice and helpful criticism.

CARTHAGE COLLEGE
CARTHAGE, ILL.

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EXPLANATION OF PLATES XVI–XIX

PLATE XVI

Fig. 2.—Thallus: *a*, side view; *b*, dorsal view.

Fig. 3.—Mitosis in apical cell; ×1850.

Fig. 4.—Median longitudinal section of apical cell; ×660.

Fig. 5.—Median transverse section of same; ×660.

Fig. 6.—Rhizoids; ×85.

Fig. 7.—Median longitudinal section of thallus through apical cell; ×250.

Fig. 8.—Same as fig. 7: *a*, young antheridium; *lf*, leaf; ×68.

PLATE XVII

Figs. 9–20.—Stages in development of antheridium.

Fig. 9.—Antheridium initial; ×790.

Fig. 10.—Young antheridium consisting of basal cell, stalk cell, and primary antheridial cell; ×790.

Fig. 11.—Vertical division of primary antheridial cell and later vertical division of stalk cell; ×790.

Fig. 12.—Appearance of transverse wall in stalk cell; ×790.

Figs. 13–14.—Formation of periclinal walls in primary antheridial cell; ×790.

Fig. 15.—Cross-section of same; ×790.

Figs. 16–17.—Division of primary wall cells; ×790.

Fig. 18.—Division of primary spermatogenous cells; ×790.

Figs. 19–20.—Older stages; ×660.

Fig. 21.—Archegonium initial and apical cell; ×625.

Fig. 22.—First division of archegonium initial, apical cell, and mucilage hair; ×625.

Figs. 23–33.—Stages in development of archegonium.

Fig. 23.—Archegonium initial; ×790.

PLATE XVIII

Fig. 24.—First division of same; ×790.

Fig. 25.—Formation of first vertical wall; ×790.

Fig. 26.—Appearance of second and third vertical walls and transverse division of basal cell; ×790.

FIG. 27.—Young archegonium consisting of primary ventral cell, primary neck canal cell, and cover cell; ×790.

FIGS. 28–30.—Formation of neck canal cells, ventral cell undivided; ×660.

FIG. 31.—Ventral canal cell and egg; ×660.

FIG. 32.—Cross-section of neck of same; ×660.

FIG. 33.—Mature archegonium; ×525.

FIGS. 34–37.—Development of embryo; ×525.

PLATE XIX

FIG. 38.—Young sporophyte; ×340.

FIG. 39.—Differentiation of spore mother cells and elaters; ×525.

FIG. 40.—Spore mother cells and elaters; ×525.

FIG. 41.—Sketch of same stage; ×50.

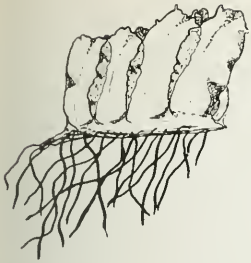
FIG. 42.—Lobed spore mother cells; ×525.

FIG. 43.—Spore tetrads; ×525.

FIG. 44.—Nearly mature spores and elater; ×525.

FIG. 45.—Sketch of same stage; ×50.

FIG. 46.—Wall of mature capsule showing thickenings on inner layer; ×790.



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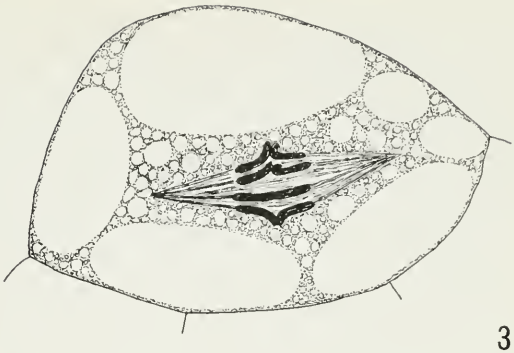

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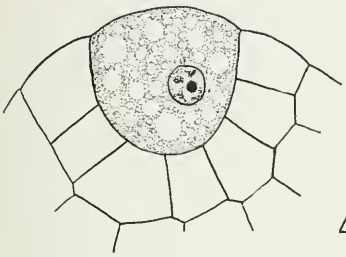

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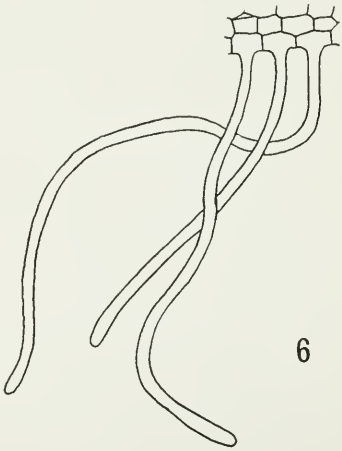

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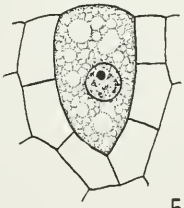

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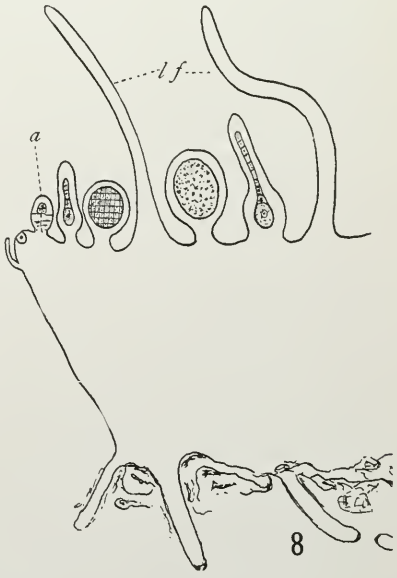

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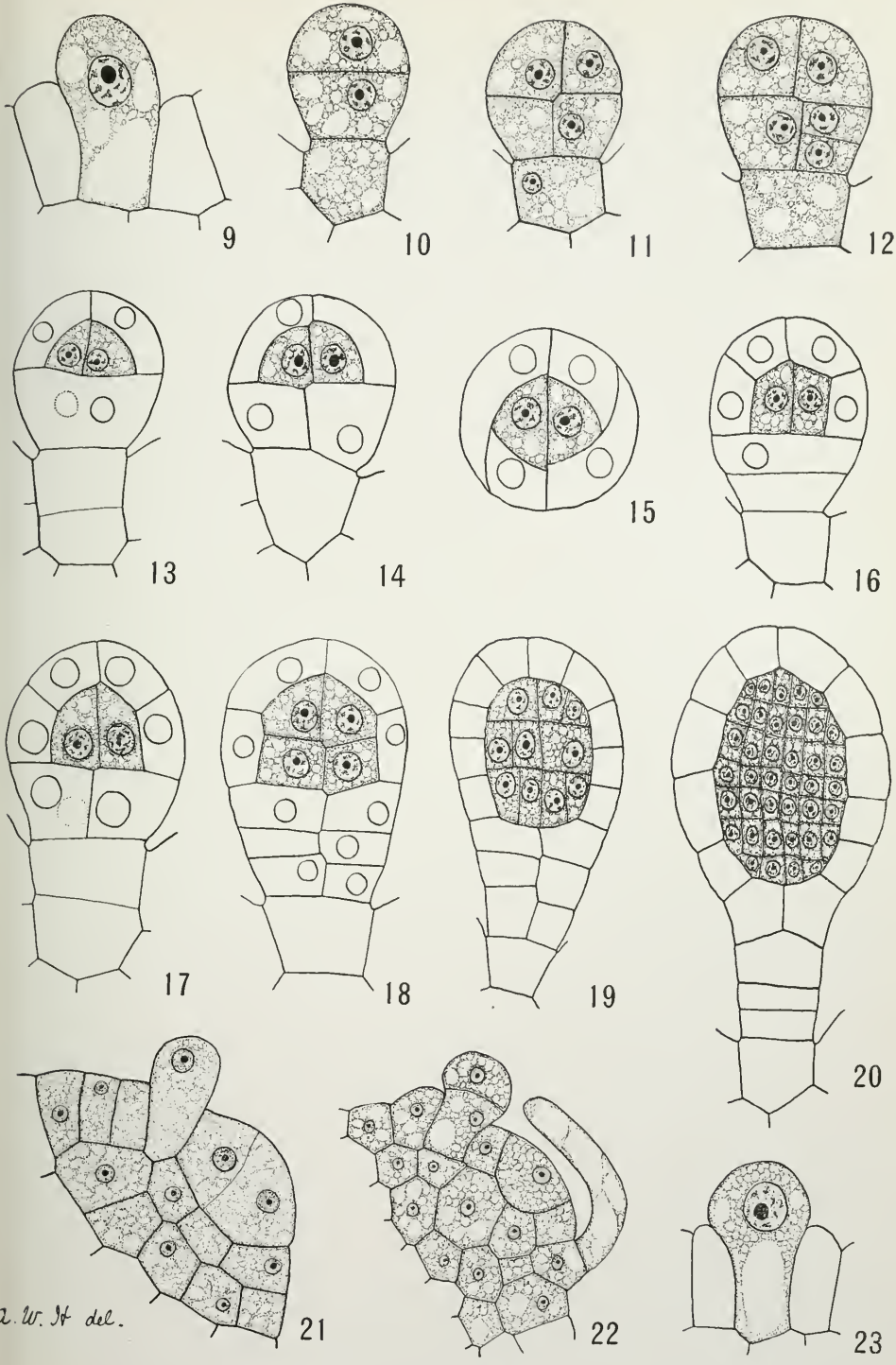

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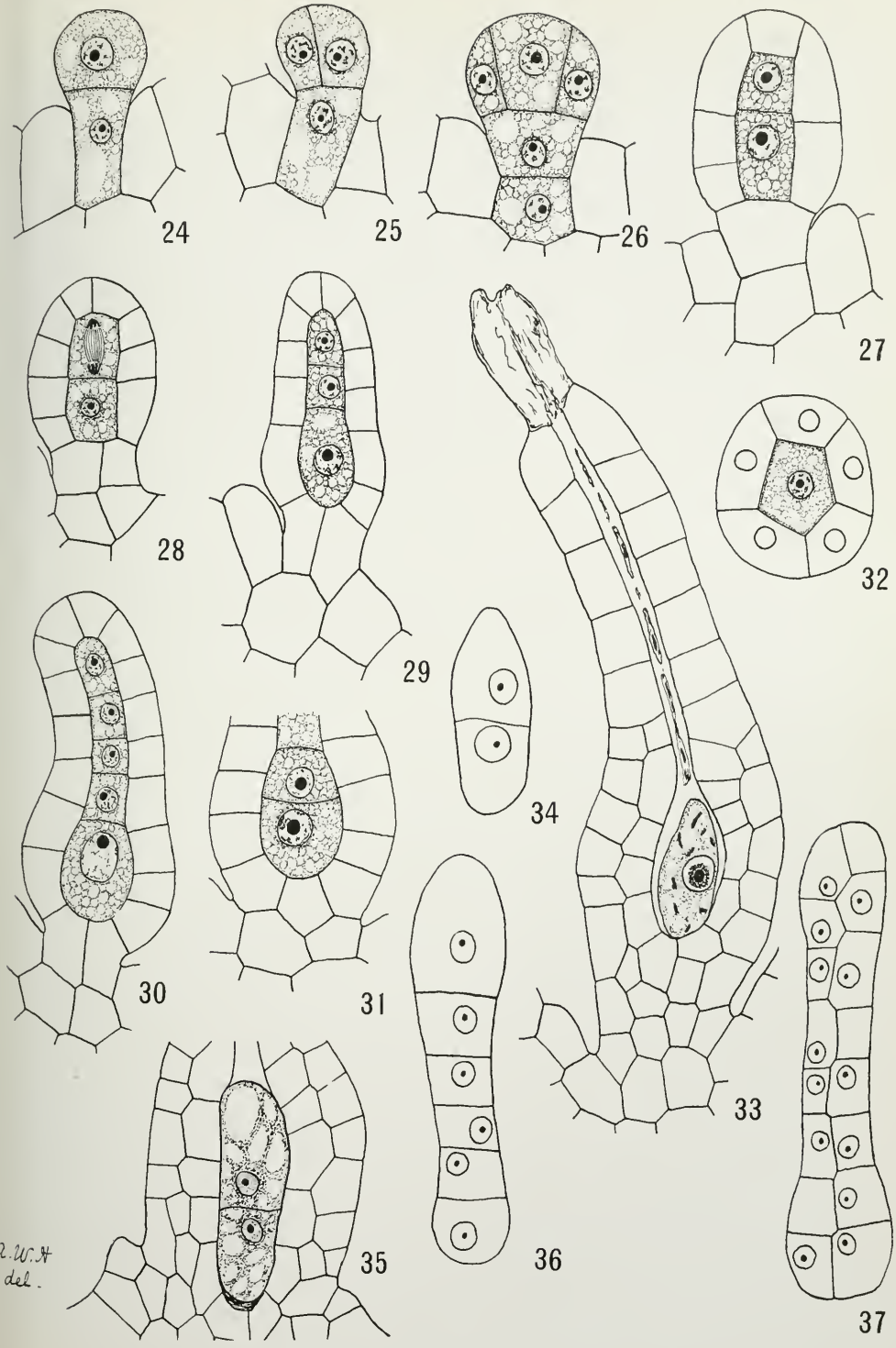

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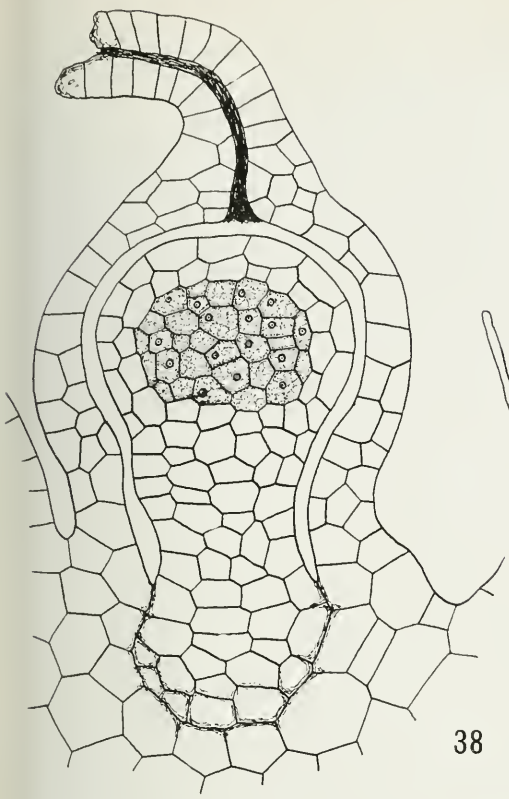
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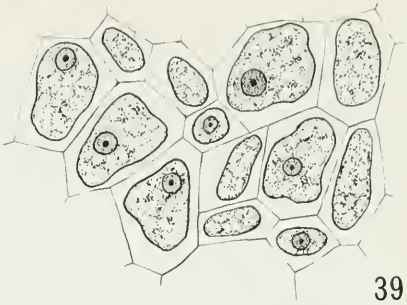
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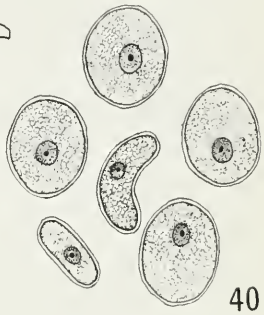
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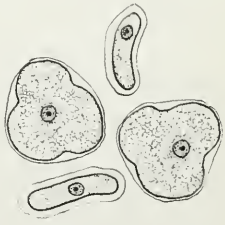
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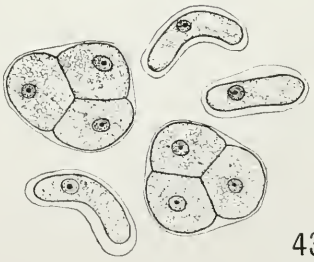
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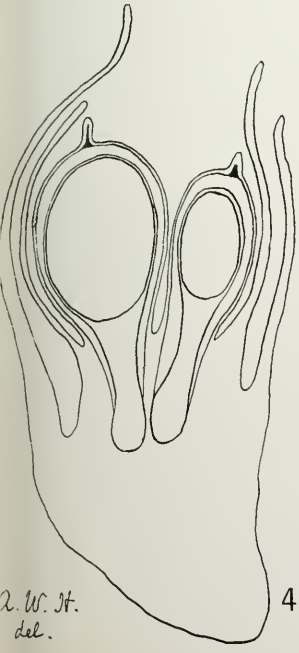

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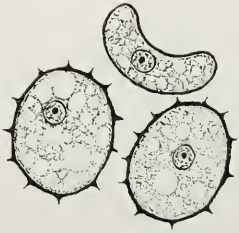
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HAUPT on FOSSOMBRONIA

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INHERITANCE OF ALEURONE COLOR IN MAIZE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 265

Merle C. Coulter

Interested in the pedagogical value of plant genetics, the writer was impressed with the fact that the bulk of our knowledge comes from experiments with corn. An investigation was undertaken, therefore, with no more definite object than to discover how dependable some of these classic experiments actually are. During three years of rather interrupted and limited investigation, this undertaking, as might be expected, has been rewarded by numerous results that have been interesting and suggestive, but by practically none that as yet can be regarded as conclusive. In view, however, of the number of investigators, professional and amateur, who are now interested in inheritance in corn, it is felt that a brief statement of a few of the results may be useful.

Technique

The writer's experiences in matters of manual technique will undoubtedly be of interest to amateurs. The grosser mechanics of corn crossing are simple and familiar. The difficulties are mainly two: (1) to avoid exposing the silks to chance foreign pollen at the time the cross is made, and (2) to insure full pollination, and hence full ears. It is common practice to remove, totally or partially, the bag which covers the silks when the pollen is applied. This involves momentary exposure of the silks to chance foreign pollen, plenty of which is almost sure to be circulating in the air. Thorough distribution of the applied pollen over the silks is then attempted by shaking the bag in some way. In the hands of an experienced operator this method is not only adequate but rapid. When the writer attempted this, however, the results were not satisfactory. Less than half the ears were fully pollinated, and there were quite a number of cases in which about 5 or 6 grains of foreign pollen had evidently been admitted. For the second season's work, therefore, a simple mechanical device was employed,

and this effectually solved the difficulties. This is a so-called corn-pollinator, a description of which has already been published.[1] Even in inexperienced hands this method will not only insure full pollination, but will never admit foreign pollen. Its drawback lies in the fact that it lengthens the process somewhat; hence it is felt that this device may be recommended to all save experienced operators, who are conducting extensive experiments.

Normal ratios

An attempt was made to discover how exactly certain complex ratios might be predicted. Material was sought in which a number of factors could interact to produce various but predictable (?) ratios. Nothing was more suitable than the set of factors involved in the inheritance of aleurone color in corn. These factors have already assumed an important rôle in pedagogy. R and C are complementary factors, the presence of both being required for the production of red aleurone, and P^2 is a supplementary factor which changes red to purple (PRC is purple, pRC red, PrC white). Other factors have been added to this set by EAST and EMERSON, but they are not dealt with in this paper. The original stock material was furnished by EAST, to whom the writer wishes to express his appreciation.

Leaving out of consideration those well established cases in which a 1:0 or 3:1 ratio is produced, it was found that the material, as originally provided, could be manipulated to produce 8 different ratios. Appropriate crosses were made, therefore, and of the resulting crop all ears containing 64 or more grains are considered in the following summary.

I.—0:1:1 (no purple:50 per cent red:50 per cent colorless) ratio predicted; from pprrCC×ppRrCC and reciprocal. Eight ears gave a total of 0 purple:559 red:659 white, or an average

[1] BOT. GAZ. 58:63. 1919.

[2] EAST and HAYES use the symbol P for this factor, while EMERSON uses Pr for what is probably the same factor.

EAST, E. M., and HAYES, H. K., Inheritance in maize. Conn. Agric. Exp. Sta. Bull. no. 167. pp. 142. *pls. 25.* 1911.

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ratio of 0:0.92:1.08. The extreme ratios on individual ears were 0:0.81:1.19 and 0:1.03:0.97. Conclusion: slight but chronic excess of colorless grains.

II.—0:9:7 predicted; from ppRrCc selfed. Five ears gave 0:418:320, or an average ratio of 0:9.06:6.94. Extreme ratios on individual ears, 0:8.77:7.23 and 0:9.45:6.55. Conclusion: predictions fulfilled.

III.—0:3:5 predicted; from ppRrCc×pprrCc. Three ears gave 0:260:430, or an average ratio of 0:3.01:4.99. Extreme ratios on individual ears, 0:3.19:4.81 and 0:2.94:5.06. Conclusion: predictions fulfilled.

IV.—1:1:2 predicted; from pprrCC×PpRrCc. Only one ear with over 64 grains obtained.

V.—9:9:14 predicted; from ppRrCc×PpRrCc. Four ears gave 229:242:262, or an average ratio of 10.11:10.35:11.54. Extreme ratios on individual ears, 10.14:12.69:9.17 and 6.51:9.83:15.55. Conclusion: decided but not chronic excess of colored grains.

VI.—3:3:10 predicted; from ppRrCc×PprrCc and pprrCc× PpRrCc. Eight ears gave 252:235:765, or an average ratio of 3.22:3.00:9.78. Extreme ratios on individual ears, 3.60:2.07: 10.33 and 4.42:3.58:8.00. Conclusions: predictions fairly well fulfilled, considering smallness of population used.

VII.—9:3:20 predicted; from PprrCc×PpRrCc. Only one ear obtained with over 64 grains.

VIII.—27:9:28 predicted; from PpRrCc selfed. Thirteen ears gave 1534:464:1560, or an average ratio of 27.59:8.35:28.07. Extreme ratios from individual ears, 23.54:9.92:30.54 and 28.97:11.03:24.00. Conclusions: predictions well fulfilled; slight tendency toward excess of purple at expense of red (as was regularly the case except, strangely enough, in the two individual ears cited under extreme ratios) may well be accounted for by improper classification, throwing a few deep red grains with the purple.

From this preliminary experiment the general conclusion was drawn that ratios produced by the interaction of these three factors were sufficiently distinct to be readily recognized in the vast majority of cases. This was confirmed by later experience,

which included also several other types of ratios produced by these same factors. Actually the only difficult distinction which was encountered was between 9:9:14 and 3:3:2, for the former (V) commonly showed a deficiency of colorless grains. How persistent and significant this deficiency is I will not venture to conclude from the limited data. Elsewhere recognition was easy so long as P, R, and C were the only factors dealt with.

Dominance

The literature is likely to leave one with the impression that dominance is complete with this set of factors. Uncertain on this matter, the writer wondered whether there was any hope of distinguishing genotypes from superficial appearance. In other words, was a grain with the formula ppRRCC a very dark red, ppRrCC or ppRRCc a lighter red, and ppRrCc a still ligher red? That such a thing might be possible was suggested by the following observation. When individuals of the formula ppRRCC were selfed, they regularly produced ears on which all of the grains were not only red but the same intensity of red. Obviously the grains were all of the same (ppRRCC) genotype. Likewise, ppRrcc× pprrCC would produce ears on which only 50 per cent of the grains were red, but always the same intensity of red. Here, also, all the red grains would be of the same (ppRrCc) genotype. In contrast with this were ears so produced that the colored grains represented more than one genotype. It was common in such cases for quite a series of color intensities to appear. Whether these different intensities to any degree represented the different genotypes was an open question, but one that could be readily decided.

An ear was chosen which had been produced by ppRrCc× PpRrCc. This particular ear was noteworthy in two respects. In the first place, where it should have shown a 9:9:14 ratio it actually gave 57:61:57. In the second place, an unusual range of color intensities appeared. Now the 9 purples theoretically should have been distributed among the following genotypes: 1 PpRRCC : 2 PpRrCC : 2 PpRRCc : 4 PpRrCc; the 9 reds, 1 ppRRCC : 2 ppRrCC : 2 ppRRCc : 4 ppRrCc. The writer therefore classified the grains on the basis of color intensity, with some

hope that four intensities of each color could be recognized. This proved impossible, since the series of intensities was practically continuous. At this point, therefore, I was forced to conclude that at least genotypes could not be sharply separated on the basis of color intensity. There yet remained the possibility, however, that color intensity might to some degree depend on genotype, the boundaries of the classes merely being obscured by individual variation. With this in view the whole series of colored grains was arbitrarily divided into several intensity classes, and these classes were planted separately and selfed.

Class W, indicating colorless, gave six large ears, all of which, of course, showed 100 per cent colorless grains.

Class R indicated faint red. This was the minimum color intensity, and may best be described by saying that a casual glance would discover no color in such grains. More careful scrutiny, however, reveals an evenly distributed (not mottled or variegated) but very faint aleurone color. In the original count of the parent ear these grains had been classified as red. Only one ear was obtained from this class, but that was very striking. Most of its grains were colorless, but some were the same faint red as the parent, and absolutely none were of any deeper intensity. Viewed at a little distance, this ear would be said to contain 100 per cent white grains.

Class R′, indicating light red, produced two ears, on which the ratios were 0:42:31 and 0:88:54 respectively. Without hesitation these were both diagnosed as 0:9:7 ratios, indicating a ppRrCc genotype.

Class R″, indicating red, produced three ears, with respective ratios of 0:76:59, 0:176:63, and 0:24:8. One feels safe in calling the first a 0:9:7 and the last two 0:3:1 ratios. The conclusion from this is that ppRrCC or ppRRCc or both have a tendency to produce a more intense aleurone color than does ppRrCc. The question arises whether the appearance of one 0:9:7 ratio from the red class was due to improper delimitation of the classes in the first place, of which there was, of course, every possibility; or whether it might have been due to inevitable overlapping of the classes owing to individual variation. I am in no position to

answer this, since I cannot say whether the particular grain which gave the 0:9:7, although included in the red class, was noticeably lighter than the other two.

Class R‴ indicated dark red (or purple?). In the original count these grains had been included with the red, but the intensity of color was such that it demanded close scrutiny to distinguish them from the purple. Four ears came from this class. Of these, one gave 0:130:33, and the other three 0:200:0 (no exact count was made of large homogeneous ears). The first was evidently a 0:3:1, with an excess of red grains, while the others obviously represented the ppRRCC genotype, the conclusions suggested being similar to that of class R″.

Class P indicated faint purple, with the same significance as R for faint red. This class gave three ears, as follows:

Faint purple	Faint red	Colorless
5	4	21
53	36	141
51	14	141

No attempt is made at present to attach any significance to these counts. In fact, it is felt that such counts are rather untrustworthy, since the color is frequently so faint as to lie at the very limit of visibility. Sufficient at present that not so much as a single light purple or light red was produced on these ears; only the faint color of the parents was regularly produced.

Class P′ indicated light purple, and gave three ears, 142:47:52, 34:14:40, and 13:1:12. The first is probably a 9:3:4 and the others 27:9:28 ratios.

Class P″ indicated dark purple, and gave one ear, 79:34:85, probably a 27:9:28 ratio with an excess of red.

The general conclusion from the preceding is that genotypes may be distinguished, to a degree, on the basis of color intensity, at least among red grains. One rather familiar with the material should be able to pick out a given genotype in most cases, particularly if he discarded intermediates, which the writer did not.

Returning now to the faint grains, one is confronted by three possible explanations: (1) these are grains, properly colored, in which an inhibitor tends to lessen materially the intensity of the

color; (2) they are grains, properly colorless, in which there is some partial substitute for the R or C (probably the latter, referred to later) factors or both; (3) they represent something entirely unrelated to the set of factors under discussion. That the second is a likely explanation is suggested by three facts.

1. The count of the original parent ear showed a marked deficiency of colorless grains. The faint red and faint purple grains had, in that count, been classified as colored. If they were truly colorless grains the original ear would have very closely approximated the predicted 9:9:14 ratio.

2. The nature of the original cross was such that every grain must be Pp or pp, but never PP. Inbreeding, therefore, might give ears with some red and no purple grains, but could never give ears with some purple and no red grains. Actually that is what occurred. This is, of course, merely negative evidence. Positive confirmation, however, comes from a similar experiment conducted with slightly different material. An ear produced by PpRrCc selfed, which gave an ideal 27:9:28 ratio, was used as the basis of an experiment similar to the preceding. The general results were about the same and need not be discussed in full. Among the purple grains, however, several produced ears which showed purple and colorless, but no red grains, and one produced a full ear of purple grains alone. This is to be expected from the fact that genotypes including PP, although not present in the previous ear, are present here. Such being the case, the behavior of the faint purple grains from this ear should prove significant. From that class came the following four ears:

Light purple	Faint purple	Faint red	Colorless
0	Some	Some	Many
0	Some	Some	Many
0	Some	0	Many
1	34	0	4

These certainly suggest the presence of PP in the last two cases, while Pp may be inferred for all the others. It is very likely that the last ear represents the homozygous condition for faint purple, whatever that may be, the color being present but indistinguishable in 4 grains.

3. EAST and HAYES (*loc. cit.*) describe in certain of their families grains which they call particolored. Their description leaves little doubt that the writer is dealing with the same phenomenon. These authors carried their work far enough to assign the P(R)c formula to these grains.

The tentative conclusion, therefore, may be reached that particolored (faint) grains lack C but contain some partial substitute. Just how this substitute is inherited is not clear as yet, but the fact that it is heritable is undoubted. It is probable, although not certain, that the same relationship between P and R as occurs in the inheritance of the normal full color maintains itself also under the particolored system. It must also be noted that this unknown substitute for C is by no means always effective in bringing any distinguishable color; its powers of expression seem to be limited by conditions, a matter which will be discussed later.

The question is now raised whether, in view of the possibility of a complete series of color gradations, reliable counts of purple, red, and colorless phenotypes can always be made. In answer one may safely state that the phenotypes stand out sharply unless particolored grains appear; the gap between light red and colorless is a wide one. Particolored grains by no means appear in all cases; the condition which brings them may or may not be present in the germ plasm. When they do appear, they do so in considerable numbers, so that a glance at the ear as a whole will determine whether or not one has them to deal with. Thereby the investigator is warned to focus sharply upon the boundary between light colored and particolored, but even under the most practiced eye some slight error is likely to creep in at this point.

An anomalous case

The possibility of at least partial substitution for the C factor has been mentioned. We may be dealing with something of the same sort in the following unusual case. EAST provided the writer with an ear produced by PPRrcc×pprrCC. The expectations were obviously fulfilled, half of the grains being purple and the other half colorless. The former, in the many crosses made, regularly revealed the PpRrCc formula, which was expected; while

the latter, in all cases but one, revealed PprrCc. In this one case the individual produced by this supposed PprrCc grain was selfed, and gave a ratio of 46:0:36. This is a perfect 9:0:7 ratio, such as would be produced by PPRrCc, but such a formula is out of the question in view of the history behind the ear. The obvious but heterodox suggestion is that some unusual condition is present, which, together with both P and C, results in purple aleurone; while with C alone it gives, not red, but colorless.

That this is a pathological case is suggested by two facts: (1) practically all the grains on this ear had their pericarps split irregularly, an unusual condition; (2) when planted, they germinated very slowly (or not at all), giving 3-inch plants at the time that all the neighboring rows had attained 3 or 4 feet; by harvest time a few small tassels had just appeared, but no silks.

Mottling

EMERSON (*loc. cit.*) has described the following situation in certain of his families. When the R factor enters the cross with the male parent only, a mottled aleurone results, while in all other cases a solid aleurone color is produced. Thus, RRcc×rrCC gives 100 per cent solid red; rrCC×RRcc gives 100 per cent mottled red; RRCC×RRcc gives 100 per cent solid red; RrCC selfed gives 50 per cent solid red, 25 per cent mottled red, and 25 per cent colorless.

The writer wishes to express his appreciation to EMERSON for providing material of the well known C tester and R tester. These races behaved with considerable regularity, for crosses between them consistently yielded 100 per cent solid purple grains when R tester was used as the male parent (PPRRcc×PPrrCC), and 100 per cent mottled grains when C tester was used as the male parent (PPrrCC×PPRRcc).

Splashed purple grains, recognized by EAST (*loc. cit.*) in most, but not all, of his families, were doubtless due to the same phenomenon. In the particular material of EAST's which was furnished, however, nothing of the sort could be identified, even in the very numerous cases in which the R factor came in with the male parent only. It was felt, therefore, that crosses between the material from EMERSON

and that from EAST would prove interesting. Evidently the former contains something which the latter lacks, and this something brings mottling instead of solid color, provided always the R factor comes in with the male parent only. Such crosses should help interpret this mottling, and should also reveal whether the P, R, and C factors of these two investigators are identical. Four crosses between C tester and EAST's material will be considered, with the families resulting therefrom.

TABLE I

EAR	CROSS	COLOR RATIO			MOTTLING RATIO		
		Predicted		Observed	Predicted		Observed
646	*PpRrCc* selfed.....	27:9:28	66:22:69	62:14:81	2:1	51:25	58:18
645	*PpRrCc* selfed.....	27:9:28	135:45:139	136:51:132	2:1	122:61	144:39
631	*PpRrCc* selfed.....	27:9:28	141:47:147	157:36:142	2:1	129:64	122:71
630	*PpRrCc* selfed.....	27:9:28	177:60:184	181:55:185	2:1	157:79	159:77
642	*PpRrcc* selfed.....	0:0:1	0:0:268	0:0:268	.....	.....	.....
643	*PpRrcc* selfed.....	0:0:1	0:0:461	0:0:461	.....	.....	.....
647	*PpRrCc*×pprrCc...	3:3:10	19:19:6	21:12:61	1:0	33:0	33:0
635	*PpRrCc*×pprrCc...	3:3:10	28:32:106	31:31:104	1:0	60:0	60:0
636	*PpRrCc*×pprrCc...	3:3:10	47:49:180	52:52:172	1:0	96:0	96:0
644	*PpRrcc*×pprrCc ...	1:1:6	8:8:49	9:9:47	1:0	18:0	18:0
652	*PpRrcc*×pprrCc ...	1:1:6	19:19:115	20:21:112	1:0	42:0	42:0
638	*PpRrCc*×PPRrCc..	9:0:7	88:0:68	74:0:72	2:1	49:25	52:22
649	PPRrCc×*PpRrCc*..	9:0:7	261:0:203	246:0:216	2:1	164:82	163:85
641	PPRrCc×*PpRrCc*..	9:0:7	159:0:123	156:0:126	2:1	104:52	106:50
640	PPRrCc×*PpRrCc*..	9:0:7	179:0:139	179:0:139	2:1	119:60	106:73
639	PPRrCc×*PpRrCc*..	9:0:7	314:0:234	313:0:235	2:1	209:104	259:54
650	PPRrCc×*PpRrCc*..	9:0:7	117:0:91	128:0:80	2:1	85:43	84:44
651	PPRrCc×*PpRrCc*..	9:0:7	224:0:174	239:0:159	2:1	159:80	161:78
648	PPRrCc×*PpRrcc* ..	3:0:5	63:0:104	63:0:104	2:1	42:21	44:19

I.—pprrCc (EAST) ×PPRRcc (C tester) gave 131:0:114 with all the purple grains mottled, according to expectations. Obviously these mottled purple grains represented a PpRrCc formula, the colorless PpRrCc. The number of this ear was 315, and its further behavior is recorded in table I, in which the italicized parent represents the immediate progeny of 315. Where mottling ratios are recorded, the solid color member always precedes (2:1 means 2 solid color : 1 mottled). Table I shows that predictions on the color ratios are fulfilled in all cases, with the possible exception of ear 638; while mottling ratios are similarly according to prediction with the possible (one feels like saying probable) excep-

tion of ear 639. It is a very striking fact that where no mottling is predicted (647, 635, 636, 644, 652) absolutely none appears.

II.—PPRRcc (C tester) ×ppRrCc (East) gave 62:0:52 with no mottling. Half the purple grains should have had the PpRRCc formula, the other half PpRrCc; the white grains, half PpRRcc, half PpRrcc. From the latter, two ears were selfed, both giving 0:0:300.

A cross was made between an individual resulting from one of the colorless grains as female parent, and ppRrCc as male parent. Since the former could be either PpRRcc or PpRrcc, we must consider both possibilities: (1) PpRRcc×ppRrCc would give 1:1:2 and no mottling (1:0); (2) PpRrcc×ppRrCc would give 3:3:10 and a mottling ratio of 2:1. The actual ratios were 48:28:78 and 76:0, satisfying 1.

Two crosses were made between individuals resulting from two of the colorless grains as male parents, and PPRrCc as female parent. The two possibilities are: (1) PPRrCc×PpRRcc would give 1:0:1 and 1:1; (2) PPRrCc×PpRrcc would give 3:0:5 and 2:1. One of the resulting ears actually gave 115:0:134 and 78:37. The color ratio might be either 1:0:1 or 3:0:5, but the mottling ratio decides the case in favor of 1. The other ear gave 242:0:278 and 126:116. There might be a slight doubt about the color ratio, but the mottling ratio decides in favor of 2.

Two other crosses in which 2:1 mottling ratios were predicted gave 73:38 and 162:65 respectively. We may conclude that this family also fulfils the predictions fairly well. It is interesting that cases in which the color ratio may be a matter of some doubt may sometimes be decided by reference to the mottling ratio.

III.—This family resulted from a cross reciprocal to the last, or ppRrCc (East) ×PPRRcc (C tester). This gave an ear containing only 13 grains, so that the ratios are without significance. The same possible genotypes are confronted as under II. One of the colorless grains inbred gave, of course, 0:0:300. A single individual, produced from one of the purple grains, was used as male parent three times, once on its own silks and twice on PPRrCc. If this individual had been PpRRCc, selfing should have given 9:3:4 and no mottling, while the cross indicated should have

given 3:0:1 and 1:1. If it had been PpRrCc, selfing should have given 27:9:28 and 2:1, the cross 9:0:7 and 2:1. Actually selfing gave 207:63:90 and 270:0, clearly indicating the former. In the two cases of the cross indicated, the actual color ratios were 411:0:127 and 360:0:129. These are 3:0:1 ratios, and satisfy, as before, the formula of PpRRCc for the male parent. The mottling ratios in these cases, however, were respectively 278:133 and 235:125. Obviously, where it was felt that 1:1 mottling ratios could be predicted with some certainty, the actual ratios obtained were strikingly close to 2:1. The writer fully realizes the care which must be exercised in classifying mottled grains. These particular ears were not only shelled (as usual), but were counted twice, using the same standards as proved satisfactory elsewhere. One is forced to conclude that these additional data represent an exceptional behavior, sufficiently decisive to be of some real significance.

IV.—pprrCC (EAST) ×PPRRcc (C tester) gave unusual data. It is unnecessary to give all of them; sufficient at present are the mottling ratios obtained. Four ears resulted in which mottling was to be expected in a 2:1 ratio. The actual ratios obtained were 155:0, 30:8, 151:3, and 177:3. Obviously the usual mottling situation is absent. The question arises whether the few so-called mottled grains were truly such. It is probable that they were not, since there have been known to occur various types of anomalous grains which may readily be confused with true mottling. Had these grains occurred on ears known to contain true mottling, they would have been included in that class. I feel justified, therefore, in the tentative conclusions that (1) the P, R, and C factors of EAST and EMERSON are probably identical; (2) mottling is due to a heritable factor (or factors) which is present in EMERSON'S C tester and absent in the material of EAST, and that this factor probably behaves immediately as a dominant, no matter with which parent it enters the cross. No attempt is made at present to explain the 2:1 mottling ratios which appeared in two cases of family III instead of the expected 1:1. As for family IV, this situation may be explained by assuming that not all of the C tester material was homozygous for the presence of the mottling factor.

The R tester material was similarly crossed with EAST'S material. The color ratios obtained in five small families fully satisfied the predictions. The mottling ratios obtained, however, were quite different from those before, and may be summarized as follows: (1) ten ears gave 100 per cent colorless aleurone, and, of course, no mottling; (2) there were nine ears in which the R factor, wherever present, had come in with the female parent; in these, therefore, no mottling was to be expected; these gave a total of 889 self-colored grains to 1 mottled; (3) cases in which a 2:1 mottling ratio might have been expected may be taken up separately for the different families. Family I, produced by PpRrCc (EAST) ×PPrrCC (C tester), gave three such ears, with a total of 467 self-colored: 5 mottled grains (1.06 per cent mottling; extremes 1.85 and 0.00 per cent). Family II gave four such ears with a total of 333:30 (8.26 per cent; extremes 12.12 and 5.52 per cent). Family III gave one such ear with 58:0. Family IV gave nine such ears with a total of 1840:23 (1.23 per cent; extremes 2.22 and 0.00 per cent, one case). Family V gave three such ears with 638:51, or 7.40 per cent mottling; but that is not all. One of these ears gave 263:10. The other two were identical with respect to both their parents, both growing on the same plant. One of them gave 237:41, the other 138:0.

One hesitates to draw any general conclusion from these data. Certainly R tester does not contain that essential factor for mottling which was present in C tester. In the event that EMERSON'S C tester and R tester were extracted fairly recently from the same parent stock, the present situation might suggest that this unknown mottling factor was an attribute of EMERSON'S R factor itself, or at least closely linked. This, however, would involve some awkward, although not impossible, assumptions to explain the behavior of mottling in those families produced by crosses of C tester with EAST'S material; for, of course, the latter did not contain the mottling factor. The situation would be somewhat simplified if it were sweepingly assumed that the mottling which appeared in these R tester families (just described) was not true mottling at all, but just an imitation. True enough, mottling is at times fairly well imitated, but these particular imitations were so like the genuine

article that the writer is very reluctant to discard them. A safe tentative conclusion would be the following. The prime requisite for mottling is that the R factor enter with the male parent only; this perhaps is equivalent to saying "that the R factor be present in the endosperm in just one out of three possible doses." Under these circumstances mottling regularly occurs when there is also present that condition which is possessed and transmitted by most C tester individuals. A different condition, occurring in R tester, favors mottling in a small percentage of the possible cases, while the conditions present in EAST's material permit of no mottling under any circumstances. The critical data should, of course, appear in the next generation.

Partial variability

The term "partial variability" has been used to indicate the variation which may occur between different parts of a single plant as regards any given character, without implying anything about the mechanism which may explain this variation. It is therefore preferable to "somatic segregation" for the present purpose. Preliminary tests of the possibility of partial variability in corn were conducted in two very simple and obvious ways. The first, so far as it went, gave such decisive and orthodox results that it may be summarized very briefly. With respect, at least, to the P, R, and C factors, pollen from suckers that had been allowed to develop was identical in crosses with that obtained from the main plant. Ears developed on such suckers were, if present, so poorly developed as to yield no adequate ratios; but ears produced on suckers that had been allowed to develop abnormally, through early removal of the main stem itself, gave the predicted ratios in all cases. These results discouraged carrying out any large scale test of this matter, at least as regards aleurone color. On the other hand, a few sporadic cases suggest that such manipulation might yield surprising results when applied to the inheritance of plant color, and particularly chlorophyll. The other method was to apply the same pollen to the silks of two of the ears on the same individual, and to compare the ratios obtained. Such attempts were frequently unsuccessful, owing to the inability of the plants

to develop two sufficiently well filled ears under the handicap of artificial pollination. Hence it was very common to get one full ear, while the other contained so few grains as to be practically worthless. This depends in part, of course, on the variety of corn used.

The bulk of the data obtained was on starchy-sweet and yellow-colorless ratios, and will be merely summarized at present: (1) the ratios were virtually identical on both ears, and (2) any marked deficiency of a given class on one ear always appeared in a strikingly

TABLE II

Ear	Count	Observed ratio	Predicted ratio
660.....	206:161	56.13:43.87	56.25:43.25
661.....	203:158	56.23:43.77	56.25:43.25
704.....	242:81	74.61:25.39	75.00:25.00
707.....	275:91	74.93:25.07	75.00:25.00
683.....	118:45	72.39:27.61	75.00:25.00
684.....	47:19	71.21:28.79	75.00:25.00
682.....	163:54	75.57:24.43	75.00:25.00
680.....	33:14	70.21:19.88	75.00:25.00
572.....	108:33	76.59:73.41	75.00:25.00
571.....	62:15	79.48:20.58	75.00:25.00
670.....	138:80	63.30:36.70	75.00:25.00
678.....	276:94	74.59:25.41	75.00:25.00
557.....	43:42	50.58:49.42	50.00:50.00
558.....	25:25	50.00:50.00	50.00:50.00

similar degree on the other ear. Relative to some of the ratios discussed earlier in this paper, representative data follow.

I. Colored-colorless ratios are markedly consistent for both ears, as may be seen from table II. The only decided inconsistency is between 670 and 678. There is little doubt that this inconsistency is real and significant, although it cannot at present be interpreted. One feels, however, like regarding this as an isolated exception.

II. Purple-red ratios are also about as consistent as could be expected (table III). Probably the only significant case is 682–680. Although the converse has been well demonstrated for other

ratios, it is altogether probable that such apparent deficiencies of red in a purple-red ratio, instead of being due merely to chance, are not only characteristic of given individuals, but heritable to some

TABLE III

Ear	Count	Observed ratio	Predicted ratio
660	155:51	75.24:26.76	75.00:25.00
661	152:51	74.39:25.61	75.00:25.00
704	182:60	75.20:24.80	75.00:25.00
707	204:68	75.00:25.00	75.00:25.00
683	89:29	75.42:24.25	75.00:25.00
684	38:8	80.85:19.15	75.00:25.00
682	132:31	80.98:19.02	75.00:25.00
680	29:4	87.87:12.13	75.00:25.00
670	101:37	73.19:26.81	75.00:25.00
678	212:64	76.81:23.19	75.00:25.00
571	52:10	80.64:19.36	75.00:25.00
572	76:32	70.37:29.63	75.00:25.00

TABLE IV

Ear	Count	Observed ratio
660	202:4	98.06:1.94
661	202:1	99.51:0.49
704	242:3	98.77:1.23
707	272:6	97.84:2.16
683	106:12	89.83:10.67
684	42:5	89.37:10.63
682	154:9	94.48:5.52
680	29:4	87.87:12.13
571	58:4	91.91:8.09
572	84:24	77.77:22.23
670	130:0	100.00:0.00
678	235:41	85.14:14.86

degree. It must be realized, however, that such deficiencies of red are probably apparent rather than real; improper classification may throw dark red grains with purple, but it may well be that the border line is thus obscured through some heritable tendency.

III. If one is to regard true mottling as limited to those cases in which the character appears in *all* the grains which receive R from the male parent only, that is, descendants of C tester, then, unfortunately, no data are at hand on comparative mottling ratios in two ears with the same parents. Such data, however, are available from descendants of R tester, where mottling (or pseudo mottling, as it may prove to be) appeared in relatively small percentages, as shown in table IV. The discrepancies apparent in table IV may be taken to indicate that the supposed mottling of the R tester line is altogether sporadic and without genetic significance. Even though that may be so, the surprising case of 670–678 is probably worthy of further investigation.

IV. Among the progeny of the faint colored grains only two poor examples of this sort are available, as may be seen in table V.

TABLE V

Ear	Count	Observed ratio
	Faint : Colorless	
562.....	17:46	26.98:73.02
563.....	26:104	20.00:80.00
557.....	0:42	0.00:100.00
558.....	9:16	36.00:64.00

It is highly probable that the occurrence of these faint grains is sufficiently limited by local conditions so that discrepancies between two such ears will prove common. This is also suggested by the results of a test which need only be briefly mentioned at present.

It is expected that the numerical value of any ratio, which is affected only by matters Mendelian, will conform with predictions based on the laws of chance. With equal certainty the laws of chance indicate that, where a 1 colored:1 colorless ratio appears, we will not find many long strings of colored grains lying together in the same row. Actually we may expect that the colored grains will be scattered within the rows of the ear in such a way that there will be, speaking relatively, n groups of 1 colored grain each, n/2 groups of 2, n/4 groups of 3, etc. These values will differ, of course, with the ratios themselves. Thus, in a 3 colored:1 colorless,

there will be n groups of 1 colorless, n/4 groups of 2, n/16 groups of 3, etc.; while, for the colored grains, there will be n groups of 1, $\frac{3n}{4}$ groups of 2, $\frac{9n}{16}$ groups of 3, etc. The exact values can easily be calculated for any ratio, although they frequently must be carried to several decimals. Such a test was applied to numerous types of ratios, and also, for purposes of control, to the tossing of coins (singly or in groups, depending on the ratio). The purpose of the test was to discover whether any of the characters under consideration were determined or limited by local conditions within the ear. Preliminary tests of this sort showed that starchy-sweet and colored-colorless ratios depended only on laws of chance. When the test was applied to the self-colored-mottled ratios of the C tester progeny, the laws of chance were fairly well satisfied, but, undoubtedly, to a less degree than in the other cases. Such slight nonconformity as occurred, however, might well have been accounted for by difficulties in classification. Tests applied to the mottling of the R tester progeny were not thought to be significant, owing to the very small percentages of the mottled grains appearing. With the particolored grains, however, very decisive results were obtained, indicating clearly that local conditions on the cob affect the appearance of this character. Thus in ears on which less than 10 per cent of the grains were particolored, the majority of the total number of such grains was made up of a few groups of 4 or 5 each. This is particularly interesting, since it has been demonstrated that this condition is heritable. The puzzle will probably be solved by finding that local conditions on the ear do not determine, but merely limit, the appearance of the particolored condition. It is expected that some, but not all, of the seemingly quite colorless grains from these ears will perpetuate the particolored tendency.

Summary

1. The use of the corn-pollinator is recommended to amateur investigators.

2. The P(Pr), R, and C factors for aleurone color, variously combined, gave predictable and readily distinguished ratios.

3. In many cases the exact genotype, with reference to the R and C factors, can be distinguished by superficial appearance.

4. The particolored grains of EAST and HAYES were identified. In these some partial substitute enables the P and R factors to express themselves, even in the absence of C. This substitute is heritable, but its effectiveness is limited by local conditions in different parts of the ear or of the plant as a whole.

5. Mottling (EMERSON), which occurs when the R factor comes in with the male parent only, is conditioned by the presence of a heritable factor or factors. This factor occurs in the C tester family, and is dominant in crosses with families which do not show mottling. The R tester family seems to contain a different factor, which produces apparent mottling only in a small percentage of the expectations.

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ECOLOGICAL SUCCESSION OF MOSSES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 266

ARAVILLA M. TAYLOR

(WITH TWO FIGURES)

The work on which the present study in moss succession is based has been confined, with the exception of that done at Mount Carroll, Illinois, to what may be termed the Chicago region. This includes localities showing the typical plant associations within about 40 miles of the city of Chicago. Since the deep rock canyon type of topography is entirely absent in this region, a study has been made of the Carroll Creek canyon, east and west of the town of Mount Carroll, which lies in nearly the same latitude as Chicago and about 125 miles west. The work was begun during the summer of 1916 and continued through the years of 1917 and 1918.

The nomenclature for the plant associations here employed is largely that used by COWLES (3) in his ecological work carried on about Chicago and other localities. Some of these terms may be traced back to WARMING (13), or perhaps farther. The first botanist to make use of this classification by which WARMING divided all plants into xerophytes, mesophytes, and hydrophytes in connection with bryophytes was WARNSTORF (14). Since that time EVANS and NICHOLS (5) have employed these terms in describing the mosses of Connecticut. The terms hydrarch and xerarch were employed by COOPER (2), and are here given the same meaning. The terminology for the classification of the moss species has

been confined wherever possible to that given by GROUT (7). The writer is under great obligation for the verification of all species and the entire classification of many to Mrs. ELIZABETH BRITTON, of the New York Botanical Gardens, Mr. GEORGE B. KAISER, curator of the Sullivant Moss Society, and Dr. LEROY ANDREWS, of Cornell University, and for many suggestions and other valuable assistance to Dr. HENRY C. COWLES and Dr. GEORGE D. FULLER, of the University of Chicago.

Description of region

The city of Chicago occupies a part of the land once covered by Lake Chicago (9). This was a post-glacial body of water formed in the depression between the Valparaiso moraine and the edge of the retreating ice sheet as it slowly moved northward. That the water remained comparatively stationary at certain levels for a considerable length of time after the recession first began, is proved by the presence of at least three distinctly defined old lake beaches. The Glenwood beach marks the edge of the Valparaiso moraine, and is the beach first formed by the impounded water; the Calumet beach was formed at a later period when the water was about 20 ft. lower than at the Glenwood stage; the third or Tolleston beach records a period when the water had receded until it was 20 ft. below that of the Calumet. The beach of the present lake is not far from 20 ft. lower than the level of the Tolleston stage of Lake Chicago, making the surface approximately 60 ft. below that of the original body of water. Going northward along the west shore of Lake Michigan one crosses, in the vicinity of Rogers Park, several old beach ridges of the Tolleston stage. Here the present lake is eroding material deposited by the older body of water. Farther north, about Winnetka and Glencoe, these old beaches have disappeared, and the lake is encroaching upon a bluff of morainic clay, where may be found all stages of clay ravines, from freshly eroded gullies to old ravines in an advanced stage of mesophytism. These ravines have their origin in the small streams which have cut back into the surrounding oak upland. Facing the lake the bluffs are in some places entirely bare of vegetation, while in others they have become well covered

with various species of trees and shrubs, such as *Thuja occidentalis*, *Juniperus communis*, and *Shepherdia canadensis*. On such stabilized bluffs, as well as in the mesophytic ravines, mosses form a conspicuous part of the ground flora. At other points north of Glencoe old dune formations are being eroded. The dune associations, however, are much better shown at the south end of the lake, so that no study of mosses on dune sand has been made along the west shore.

At the south end of Lake Michigan is an extensive sand dune formation reaching southward for some distance. The finer particles of the material eroded on the west shore are carried by the water currents toward the south and there washed up on the beach. The prevailing winds blowing from the lake catch up this fine sand as it becomes dry and carry it farther inland, thus continuing year after year the process of dune building (3). At almost any point which has been left undisturbed by man may be found all stages, from the bare foredune, through the series of cottonwood, pine, early oak, and the well established mixed oak dune formations. At Miller, Indiana, where a part of this work was carried on, the pine dunes are especially well illustrated to the south and east of the Grand Calumet River. This stream, which rises in eastern Indiana and flows almost directly west as the Little Calumet, makes an abrupt curve south of Calumet Lake. It formerly flowed eastward as the Grand Calumet, in a course nearly parallel with that of the Little Calumet, to its outlet into Lake Michigan north of Miller. Later, sand dunes began to form across the mouth, and the stream, being extremely sluggish, was not able to remove the accumulating deposit and was forced to find a new outlet, its present mouth near South Chicago, thus following the path of least resistance. The Grand Calumet now remains as a nearly stagnant body of water which is rapidly filling up with typical pond vegetation. The dune slopes south and east of this part of the river form one of the best moss habitats to be found in the dune complex. Much of the natural flora near the lake and along both banks of the river is being destroyed by the building of cottages. The level of the water in the Calumet has been raised by a dam recently built across the stream farther west. This has not only flooded the low

marshy land near the old outlet and many of the pine pannes north of the river west of Miller, but has submerged the lower part of a transition oak-pine slope south of the river where a special study of mosses had been begun in 1916–1917. Another similar, but perhaps somewhat more mesophytic, habitat is found near Tremont, Indiana, several miles east of Miller on a slope approximately at the same distance from the lake, and south of a smaller stream, Dune Creek, which also flows nearly west for some distance and here empties into Lake Michigan. This also shows a transition from the conifer to the deciduous type of trees, but contains some more mesophytic species, such as *Liriodendron Tulipifera* and *Acer saccharum*, not found at Miller. Mosses are even more abundant here than on the transition slope along the Calumet.

In addition to the region about Tremont and Miller the dune formations have been studied also at Paul, Pine, Long Lake, and Buffington, all located in Indiana. In all these places the same general conditions are met. Starting at the Lake Michigan beach and going southward may be found, in fairly regular order, first the foredune and cottonwood dune on which there is almost constant shifting of sand, followed by the slightly higher and more nearly established pine dune. This is often succeeded by a transition region of mixed oak and pine which merges into the oak dune proper, so that the oldest of the series and the one farthest from the lake is that of the established mixed oak dune on which *Quercus alba* and *Q. velutina* are dominant. These older dunes lie on the border line between the beech-maple climax region of the eastern United States and the oak-hickory climax which seems to be typical near the Mississippi River. For this reason it is somewhat difficult to determine whether these oak forests belong to the latter climax type, or are subclimax associations which will in time develop into the beech-maple type (3).

South of the dune complex just mentioned is another interesting type of topography very completely described by SHELFORD (10). This is a low swampy area made up of long shallow ponds or lagoons, nearly 100 in number, separated by ridges and extending almost parallel to the present lake shore. These ridges were formed by the building up of barrier beaches along the former

shore line, thus cutting off portions of the lake, which then became lagoons. At one time these ponds drained either into the Calumet River or directly into Lake Michigan. Much of this drainage has been cut off by railroad embankments built across the ridges and lagoons, so that these depressions now exhibit a characteristic pond flora. Some of the ponds have reached the shrub or swamp-forest stage; others are dominated by an almost pure stand of cat-tails or bulrushes; still others, ecologically younger, have a considerable area of open water. The ridges in most cases are covered with oak forests.

In addition to the lagoons, hydrophytic habitats are to be found in various swamps and bogs which lie within the Chicago region, all of which offer excellent conditions for bryophytic development. These may be divided into two main types, those which have developed from deep kettle lakes and those which have been formed from shallow lakes or ponds. The former type is illustrated by the bogs at Mineral Springs and Hillside, Indiana; while the latter is represented by the swamp forests at Thornton, Illinois, and Furnessville and Wilhelm, Indiana. The Mineral Springs bog has been developed by marginal encroachment of vegetation on the bottom and by formation of a surface mat in which *Decodon verticillatus* has played an important part. The progression has passed beyond the open water of floating vegetation stage, and even the early stages of mat formation seem to be somewhat telescoped; but here and there are small areas in which either the cat-tails, the bulrushes, or the sedges are dominant. This fen association merges into the shrub stage in which *Rhus vernix*, *Cephalanthus occidentalis*, and *Alnus incana* are most abundant. Beyond the shrub association is the tree area with *Larix laricina*, where in places the quaking condition is still quite evident. In the drier portions of the forest *Betula lutea*, a tree rare in the vicinity of Chicago, makes its appearance. The Hillside bog seems to have had the shrub stage, which here comes in on a dense growth of *Sphagnum*, continued until the substratum is comparatively dry, the forest stages having been subjected to a much greater retardation than is the case at Mineral Springs. The other swamps mentioned have been produced by marginal

growth of plant life on the bottom only. The Thornton swamp lies directly south of Chicago and between the Valparaiso moraine and the Calumet beach line. The Furnessville swamp is east of Mineral Springs, and at about the same distance from Lake Michigan. Both of these swamps have reached the forest stage of development, although there may be standing water in the depressions in the early part of the season. The third swamp, that at Wilhelm, is ecologically of a more advanced type. There is little standing water at any time, and the trees (oak, beech, and hard maple) indicate the approach of the climax forest.

Nearly all of the other associations under consideration are located on morainic drift, either within the region once occupied by Lake Chicago or on the moraine forming the uplands about its borders. Within the Chicago Lake area this till material has been somewhat worked over by water action, but not to a degree sufficient to entirely destroy its drift character. On the east bank of the Des Plaines River, just below its junction with the Sag, is the town of Lemont, Illinois. Here there is an outcrop of limestone which forms several small rock ravines. An abandoned stone quarry in the vicinity, as well as a stone wall at Palos Park and a quarry at Thornton, offer very similar pioneer rock surface habitats. East of Lemont near Palos Park on the edge of the Valparaiso moraine is an upland oak forest which is probably a subclimax forest. Excellent secondary successions in cut-over oak forest in various stages toward reforestation are found south of Lemont near Joliet. East of Joliet along Hickory Creek near New Lenox are much more mesophytic oak-hickory upland forests. At other places we find climax forests of the beech-maple type. At Smith, Indiana, a few miles east of the Wilhelm swamp forest, and at Otis, Indiana, southeast of Chicago, are primeval woodlands containing beech and hard maple of very large size, placing them without question in the climax area of the eastern United States. Along the Des Plaines River south of the northern boundary of Cook County, near Wheeling, Illinois, are mesophytic forests on uplands in which the presence of *Acer saccharum* indicates a greater degree of mesophytism than is frequently met with so far west in northern Illinois. No *Fagus grandifolia* has been

found in this region; but the maple may herald the coming of the climax forest of beech and maple. Directly east of Wheeling, along the lake shore at Glencoe, the upland forests are dominated by oak, although maple is present in the ravines.

The Carroll Creek canyon is a narrow valley with high and in many places nearly perpendicular walls of limestone. The stream meanders back and forth across the ravine and frequently washes against the rock wall. All successions, from the first pioneer lichens and liverworts to trees with decidedly mesophytic undergrowth, may be found within a short distance of each other. This is by far the best moss habitat which has been included in the present study. Although no evaporation data are available upon this region, it is probable that the excess of humidity over evaporation is greater than in the Chicago region proper; while the absence of dust from factories and smokestacks may also be a factor in favor of more luxuriant moss development.

Plant successions

All the successions studied may be placed in two general groups, xerarch successions and hydrarch successions.

Xerarch successions

Under the xerarch series are included all successions which have developed from or through xerophytic stages even though not xerophytic at the present time. Among the most important of these within the Chicago region are the successions on dune sand.

Sand dune succession.—The lake beach, while not strictly a dune formation, must necessarily be included in the dune series leading back from the lake. Here the sand is constantly being moved, either by the waves or, when dry, by the wind. Even during the summer the waves frequently wash over a space several rods in width; while in winter the effect of water and ice is felt still farther inland. Very few plants are able to gain a hold under such unfavorable conditions. Occasionally a few annual seed plants can be found; and sometimes upon the upper beach seedlings of the cottonwood and willows, as well as a few grasses, begin

a precarious existence. Mosses are entirely absent, no evidence having been found even of early germination stages. In addition to the continual change in the surface there is exposure to high evaporation, another factor very unfavorable to plant life.

The foredunes are a result of the obstruction offered to the sand laden winds by plants or other obstacles. Among the plants which may act as windbreaks are *Populus deltoides*, *Prunus pumila*, *Salix glaucophylla*, and *Salix syrticola;* or grasses, as *Ammophila arenaria* and *Calamovilfa longifolia*. There is no indication that mosses ever form a part of the flora. Exposure to evaporation and danger of smothering by sand are probably nearly or quite as great here as on the beach itself. As we enter the cottonwood dune, which is the first of the dune series characterized by trees, we still find constant shifting of sand. Evaporation, however, because of the shade cast by the trees, is somewhat less than in the earlier association. Gradually the sand increases in height about the trees, which continue to grow by adventitious roots (3). In time deposit of refuse from the cottonwoods and growth of ground flora add to the humus content as well as lead to stabilization of the sand. Occasionally under the larger trees or on the more protected leeward side of the dune a few mosses may win out in the competition and live. The first species to appear are such xerophytic forms as *Ceratodon purpureus*, *Bryum ventricosum*, and *B. caespiticium*. If well sheltered, these mosses may continue on into the *Pinus Banksiana* association; or if exposed by change in direction of wind, may be entirely killed out before the cottonwoods are replaced by pines. In no place on the cottonwood dune does there seem to be any considerable growth of mosses. The species mentioned form only scattered tufts or cushions, although in most cases sporophytes are borne freely. Either germination of spores does not often occur, or the young plants do not survive the unfavorable environment. These species probably do not spread so readily by vegetative growth as do many others.

From the cottonwood to the pine dune we usually find a gradual transition, in which *Pinus Banksiana* begins to appear more and more abundantly until the cottonwoods have been eliminated. At about this time *Pinus Strobus* becomes mixed

with *P. Banksiana* on the more mesophytic slopes, and eventually may form a pure stand. During even the early pine stages we may find a thick undergrowth of *Juniperus communis*, with or without *Arctostaphylos Uva-ursi.* These may last until the oaks begin to encroach upon the pines. Both the juniper and the pines produce a dense shade throughout the year, and by shedding needles form a layer of slowly decaying débris. Under the juniper, particularly on north facing slopes, we find the most abundant moss growth of the dune series. Beyond the juniper, where *Arctostaphylos* is very thick, mosses may be present but are less continuous. The bearberry is a plant of low trailing habit, and has the effect of shutting out the relatively small amount of light which penetrates through the dense covering of conifers, and renders photosynthesis on the part of the mosses difficult. The most abundant species of moss under the juniper is *Thuidium delicatulum*, ordinarily considered very mesophytic. Here it forms a thick continuous mat frequently excluding all seed plants as well as most other moss species, and extending beyond the juniper in many places. In this moss mat is a much smaller quantity of *T. recognitum*, not mixed with the *T. delicatulum* but growing in similar places and forming small but distinct portions of the mat. A still smaller amount of *T. abietinum* appears occasionally. Scattered through the *Thuidium* in very small quantities are two other mesophytic species, *Hylocomium triquetrum* found at Paul, and *Calliergon Schreberi* found at Miller. Both species are common in the mesophytic forests farther north (2). About 15 other species of mosses occur upon the pine dune. Some of these are found occasionally under the juniper, but more often on the sand in open places free from juniper, around the bases of trees, or on half-decayed sticks. The most common of these are *Ceratodon purpureus*, *Dicranum scoparium*, and *Funaria hygrometrica*, all of which are species of fairly varied habitat. Much the same condition has been found in all of the pine dunes studied. The mosses are most abundant in total quantity and are most luxuriant on north facing slopes, which in this region are also lakeward facing slopes. A greater number of species occur here than elsewhere in the dunes, unless it is in the transition oak-pine regions, where many

of these species continue on as relics while new ones make their appearance.

Just west of the pine dunes at Miller and south of the Grand Calumet is such a transition region of mixed pine and oak. Along the slope near the river is an abundant growth of mosses, but nowhere except close to the water do they form as complete a covering as in the pine association. Toward the top of the slope they become scattered, and there is also a decrease in the number of species. *Thuidium delicatulum* continues on the lower slope with some *T. recognitum*. Other types found among the conifers are mixed with new species, one of the most common of which is *Fissidens cristatus*. Other forms, either new or now much more abundant, are *Mnium cuspidatum*, *Thelia Lescurii*, *Anomodon rostratus*, *Climacium americanum*, and *Rhodobryum roseum*.

As mentioned previously, another ecologically more advanced transition slope occurs south of Dune Creek near Tremont, Indiana. Conditions here are even more favorable for mosses than at Miller. The presence of such trees as tulip and hard maple before the pines are entirely gone would indicate a telescoping of the oak stages and the rapid advance of the climax forest. The same relative difference in scattered moss patches on the upper slope and almost continuous mat near the base is noticeable here as at Miller. The most conspicuous species is *Aulacomnium heterostichum*, bearing numerous sporophytes. Other mesophytic species not mentioned before are *Bartramia pomiformis*, *Catharinea undulata*, and *Dicranella heteromalla*. *Anomodon attenuatus* occurs in dry situations, usually on tree bases. As already mentioned, both of these transition slopes are near the lake, north facing and south of streams. In striking contrast to these are transition slopes directly south of the pine dunes, farther from the lake, and not in close proximity to streams. Here we see a rapid thinning out of the moss flora. The more mesophytic species disappear entirely and only a few new forms come in. These resemble the types found at the xerophytic tops of the more mesophytic transition slopes.

In the early stages of the oak dune proper, either farther west along the Calumet or south of the pine dunes at Miller as well as at Paul and Furnessville, the mosses are still scattered. In

ravines, however, on slopes with a northern exposure or otherwise protected from desiccation, certain species may be fairly frequent. *Thelia Lescurii*, a gray-green moss growing in loose mats, is dominant and sometimes covers areas of several square feet. *Anomodon rostratus* also appears frequently, and *A. attenuatus* occasionally. *Climacium americanum* and *Rhodobryum roseum* may be found in sheltered spots but not in large quantities. *Ceratodon purpureus* is characteristic in open, less shaded places, while *Catharinea undulata* occurs here and there. A thick continuous moss carpet is never found among the oaks as in the pine

TABLE I

PRESENCE OF MOSS SPECIES IN ASSOCIATIONS OF SAND DUNE SUCCESSION

Species	Cottonwood	Pine	Transition pine-oak	Oak	Beech-maple
Anomodon rostratus	.....	P	P	P	.....
Bryum ventricosum	P	P	.....	.....	.....
Bryum caespiticium	P	P	.....	.....	.....
Catharinea undulata	.....	P	P	P	P
Ceratodon purpureus	P	P	P	P	.....
Climacium americanum	.....	P	P	P	.....
Fissidens cristatus	.....	P	P	P	.....
Funaria hygrometrica	.....	P	P	P	.....
Leucobryum glaucum	.....	P	P	P	.....
Mnium cuspidatum	.....	P	P	P	.....
Rhodobryum roseum	.....	.....	P	P	.....
Thuidium delicatulum	.....	P	P	P	.....
Thuidium recognitum	.....	P	P	P	.....
Thuidium abietinum	.....	P	P	.....	.....
Thelia Lescurii	.....	.....	P	P	.....

dune. As we go still farther south into the later stages of the oak associations, the moss flora becomes less, until about the only species left are *Thelia Lescurii* and *Catharinea undulata* in shaded places, with *Ceratodon purpureus* and rarely *Bryum argenteum* where the sand is more exposed. In forests where white oak is dominant and the forest floor is free from fallen trees, as is the case in many oak forests in this region, *Catharinea undulata* is usually the only moss species to survive. Table I shows the succession of mosses as they have been found in the xerarch series of the sand dunes. P indicates presence of species. Only the species which occur in two or more associations are included.

Why is it that we find this great variation in the moss flora within such a relatively small area as that included within this dune complex? There seem to be at least three causal factors which are worthy of special consideration. First is the constant transportation of sand; second, the exposure to high evaporation; third, and in this case of least importance, competition with other plants. Mosses, because of their low growing habits, are not able to endure covering. Even with such a genus as *Sphagnum*, which is able to continue upward growth year after year, and which has tall erect stems, it is not unlikely that a deposit of sand or sediment would entirely destroy its power of regeneration. There is much less probability that other species which do not have this advantageous habit could contend successfully against covering. Numerous places occur within this region where, through rejuvenation of some dune area, the sand is being carried over more or less mesophytic regions. North of the Grand Calumet near Miller are dunes which have reached the pine stage and which contain many of the species of moss found in the pine dunes south or east of the river. Recent changes, largely due to man, have brought about rejuvenation of the dunes to the windward. The mosses are now in many places early destroyed by smothering, because of the fine sand accumulating about them, and the whole slope, once mesophytic, is undergoing a retrograde succession. Thus it seems quite certain that any dynamic condition which will lead to covering will also bring about the death of any mosses already existing, as well as preventing the growth of the pioneer species. Contrary to the once common opinion, the soil of the new dune is not dry, except near the surface. The water table is always high, and it is necessary only to remove a thin layer of sand to find moisture, even during dry weather. The exposure to evaporation may be great, and this without doubt is the leading cause of the xerophytic structures to be found in dune plants, rather than non-availability of the water supply (6). The work of FULLER gives data upon evaporation in the dune associations, secured in this same region north of Miller. The results regarding the difference in the evaporation rate verify in a marked degree the conclusions to be drawn from the location of the xerophytic

and mesophytic types of moss. Stations for the location of the atmometers were selected in the cottonwood, pine, and oak associations near Miller, and for the beech-maple association at Otis, Indiana. The last, however, is upon morainal clay and not on dune sand. It is not necessary to enter into a detailed account of these results. Fig. 1, taken from FULLER'S work, shows the average of the mean daily evaporation rates in these associations for the three seasons 1910, 1911, and 1912. Fig. 2 indicates the curves for the average of the mean daily evaporation rates in the four associations for the growing seasons of these years.

The absence of mosses on the beach and the foredune is due to the continual change in the surface material and the exposure

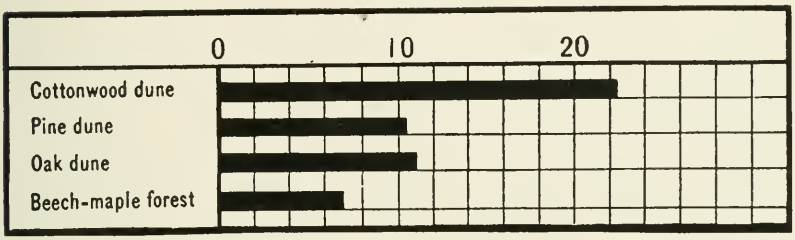

FIG. 1.—Average of mean daily evaporation rates for the 4 associations for seasons 1910, 1911, 1912.

to evaporation. Competition with other plants does not enter into the question. There is not the struggle with wave action on the foredune as on the beach, but there is still constant movement of sand by winds. The plants forming the nucleus of the foredune cast little shade, so that both desiccation by sun and wind and the probability of being covered by sand are as great as on the beach below. The cottonwood dune is higher, the trees afford much more shade, humus begins to accumulate, and as the dune tends toward stabilization there may be much greater protection from wind on the leeward side. However, even on a moderately windy day fine sand is deposited over the ground vegetation so that there is still the struggle to overcome the tendency to covering, and for opportunity for photosynthetic work on which the life of the mosses depends. Evaporation by exposure to bright sunlight and strong winds, while still high, may be somewhat less than on

the foredune. All of these causes tend to exclude any but the most hardy species, and even these are never abundant. The

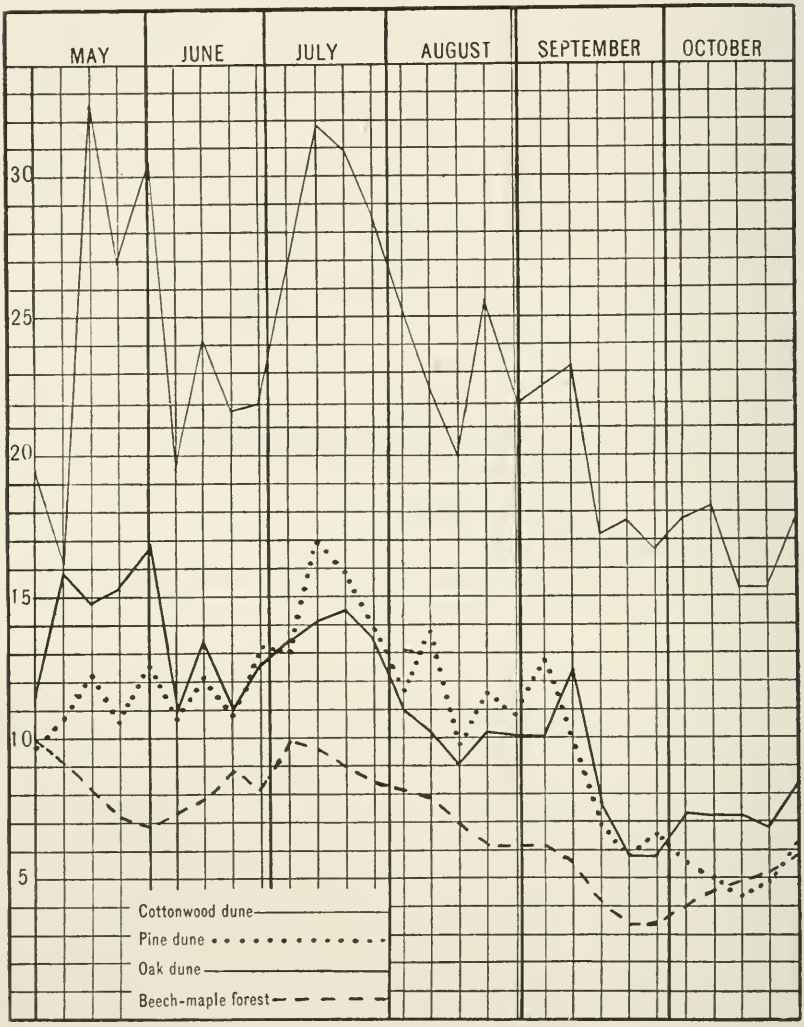

FIG. 2.—Average of mean daily evaporation rates in the 4 associations for growing seasons 1910, 1911, 1912.

struggle with other plants is not important, since there are always many unoccupied places, and the supply of available moisture is plentiful.

In the pine dune there is a much greater difference in the effect of the first two factors, moving sand and evaporation. It is here and in the mesophytic transition regions that the third factor enters into the causal conditions. According to the results of the evaporation work done by FULLER, the pine dune shows the lowest evaporation rate to be found among the tree associations of the dune series, other than the climax forest. It is still more significant that the rate is lower during the early summer and late fall, the most vital part of the season for mosses. The débris upon the ground aids in the absorption of moisture during rains. The moisture as it slowly escapes from the soil is confined near the surface by the close canopy of the juniper, and also by the dense overhead covering of pines. All of this leads to a high degree of humidity during spring and autumn, the seasons of greatest rainfall, not found elsewhere in the dune associations. In midsummer evaporation may surpass that of the oak dune (fig. 2), but the mosses by that time have passed their period of vegetative growth, and in many cases the production of sporophytes also. The maturing of sporophytes in other species, such as *Thuidium*, is carried on late in the season when humidity again rises. The fact that we find *T. delicatulum* as the dominant species under the juniper indicates decidedly mesophytic conditions, for except as a relic this species usually occurs only in moist habitats. Another reason for its dominance seems to be its ability to endure shade. Either there is no competition with other plants under the juniper or such plants have been crowded out, while *Thuidium* thrives best when well shaded. Other plants become competitive beyond the juniper where herbaceous vegetation, including several typically northern species, becomes more frequent. *Thuidium* less often covers extensive areas, and seed plants may even be found germinating on the mosses. In places more favored by light the mosses are likely to lose out altogether or be forced to take refuge on sticks or bases of trees. Another factor which seems worthy of consideration is that *Thuidium* grows directly on the slightly decayed needles of the conifers. These probably produce a chemical condition of the soil which effectively eliminates many other plants. While the pines also shed their needles, there is much

less material of this kind where the juniper is absent. The competition with shifting sand is nearly absent unless the dune is being rejuvenated. The deposit is so slight that it does not seem to retard either the germination of spores or spread by vegetative growth.

The two mesophytic transition regions from conifer to oak offer nearly as favorable moss habitats as do the pine slopes. Many of the species are relics from the more shaded former conditions, but which now are losing out, largely it would appear by encroachment of other light tolerant mosses, rather than because of competition with herbaceous plants. The shade is much less, especially during late fall and early spring. Many of the mosses are scarcely evident during midsummer. Most of them produce many sporophytes and mature the spores early in the year. That the relative humidity is at times increased by nearness to the water was quite evident on several trips to Miller when the weather previously had been warm enough to raise the temperature of the water of the Calumet. A strong cool wind from the north carried the mist, which was ascending from the river, directly over the transition slope. It was not learned how frequently this happens, but a considerable amount of moisture must be deposited during even a few hours of such a mist. This difference in humidity and water supply is probably one of the chief causes of variation in the luxuriance of the mosses on these slopes and on those farther from the lake, and not in the vicinity of other bodies of water. The evaporation rate at other times is very likely higher than on the pine dune, but unfortunately there are no data for evaporation on these transition slopes. Neither competition with other plants nor movement of sand is a very important factor, unless it may be the latter near the top of the slope.

On the oak dunes we again have an evaporation rate higher than that of the pine dune, except in midsummer. The sparse undergrowth in many places gives little protection from the hot sun which penetrates through the foliage of the oaks. During the spring and fall there is great exposure to somewhat desiccating winds. On many of the more mesophytic northward slopes where mosses might be expected there is often a dense growth of vernal herbaceous plants which seem to have crowded

out the mosses, until the latter are found only on decayed sticks or bases of trees. A few relics from the pine association occur here and there. On some slopes and in ravines where herbaceous forms have not taken full possession, mosses are more common. As previously mentioned, these are somewhat xerophytic species which appeared only rarely in the earlier succession, together with some relics from the former association. It is possible that the roots of the herbaceous plants, because of the need for moisture, rob the surface soil of its water and thus make it more difficult for mosses to secure a sufficient supply. Competition, therefore, can be said to be the great limiting factor on the more mesophytic slopes; while low humidity and high evaporation seem to be more important on those facing the south, where neither mosses nor herbaceous plants are very abundant. Sand laden winds are not of much importance unless the area is near a rejuvenating dune. In the older stages of the oak succession the forest becomes more mesophytic. There is less evaporation and higher humidity, with entire lack of covering by sand. Humus has now accumulated to a degree necessary for the growth of many more species of seed plants. Apparently these have become so successful as to cause almost total elimination of the mosses, which have contributed to their own extinction by adding to the humus content. Only in exposed paths or roads, on decaying logs, or sometimes on tree bases, do the mosses continue to exist at all. Old logs are rare in these woods, and only bases of old trees are favorable habitats, so that in the advanced oak association in this region the moss flora is often almost confined to a few species which spring up in paths or tracks left by the feet of animals.

We may summarize the causal factors for presence or absence of mosses in the dune succession as follows. Mosses are excluded from the flora of the beach and foredune by great exposure to desiccation and to covering by sand. Xerophytic species may appear on the cottonwood dune, but are prevented from becoming conspicuous by these same two factors. Mosses suddenly become abundant in the pine dunes, their growth being favored by high humidity and low evaporation during spring and fall, a result largely of the shade cast by the pines and juniper. Competition

with other plants begins, but is not of great importance; while that with shifting sand has nearly ceased. Whether the moss flora of the transition conifer-deciduous regions resembles more nearly that of the former or of the latter type seems to depend chiefly on local conditions, such as adjacent bodies of water and exposure to winds, greater humidity tending to increase the growth of mosses, and a high evaporation rate bringing about their destruction. In the oak dunes the higher evaporation leads to elimination of the relic species, while it may also lead to the appearance of new xerophytic types. Competition with other plants, especially vernal herbs, becomes a deciding factor, while that of moving sand may be omitted from consideration.

Morainal clay successions.—The early stages of moss succession on morainic drift were studied near Glencoe, Illinois. On newly eroded bluffs along Lake Michigan mosses are absent, and in fact do not appear until after other vegetation has begun to take possession and the surface is no longer subject to very active erosion or slumping. On slopes partly covered with *Juniperus communis*, with or without *Thuja occidentalis*, mosses, while conspicuous, do not form a mat of large extent. The species are almost identical with those on sand at Miller. *Anomodon rostratus*, *Thelia Lescurii*, and *Thuidium delicatulum* are the most common. The same similarity on dune sand and morainic clay bluffs has been noted by Cowles (3) for the higher plants. Neither do mosses appear in the early stages of ravines while vertical erosion is active. In later stages, however, they become important and may take no inconsiderable part in stabilization of the surface. Unfortunately it was not possible to study ravines of all degrees of mesophytism, so that the exact period at which mosses appear was not determined. Most of the work was done in ravines having sides of rather gradual slope covered with a subclimax forest and mesophytic undergrowth. A vertical succession, not so evident on the dune slopes, is here a noticeable feature. In one such ravine *Polytrichum commune* is conspicuous among the arbor vitae at the top. Just below this is a good display of *Catharinea undulata*. About midway down the slope is a mixture of mesophytic species such as *Bartramia pomiformis*, *Dicranella heteromalla*, *Anomodon*

rostratus, and *Mnium cuspidatum;* while the lower third of the slope is nearly covered by one hypnaceous species, *Plagiothecium deplanatum*. The entire surface is well supplied with herbaceous undergrowth, but this has not yet been able to supersede the mosses, which, because of absence of decaying woody material, are found almost entirely on the ground. As the ravine widens and enters upon its second period of denudation, more light enters, and the mosses are gradually eliminated by their being a favorable habitat for the germination of seedlings of higher plants which can endure a greater degree of evaporation.

The oak uplands adjoining these ravines are characterized by an extremely impoverished moss flora with the exception of *Catharinea undulata*, which may occur frequently. This is almost equally true of the oak-hickory morainal forests at Joliet, New Lenox, and Palos Park. *Catharinea undulata* is present in all, *Physcomitrium turbinatum* occurs along paths, and at Palos Park *Leucobryum glaucum* is an occasional species. At Wheeling, Illinois, just west of Glencoe on the Des Plaines River, are upland morainal forests which are much more mesophytic than those just mentioned. Of these we may make two general divisions: those which have been pastured so that there are few shrubs and the herbaceous growth is almost confined to grasses, and those which have a mesophytic undergrowth both shrubby and herbaceous. In the unpastured woods, as a marked contrast with the other oak woods just mentioned, mesophytic mosses are common both on logs and on the ground. Among these are *Thuidium delicatulum*, *Mnium cuspidatum*, *Catharinea undulata*, and *Climacium americanum*. In the more open woods which have been partly cut over and subject to grazing, these same species continue on as relics, but are less abundant than before. With these may be *Leucobryum glaucum*, *Dicranum scoparium*, *Polytrichum commune*, and *Ceratodon purpureus*. It is not unusual to see rather large areas given over to *Leucobryum* and *Dicranum* alone or mixed with *Polytrichum*, *Catharinea*, and *Thuidium*. Close to the river, however, along the well drained bluff, we once more find only *Catharinea* on mounds and *Physcomitrium* with sometimes *Funaria hygrometrica* along paths and in tracks.

What is probably the ultimate forest of the region and the climax of the morainic series, the beech-maple type, is seen at Otis and Smith, Indiana. No mosses except *Catharinea* have been found in these forests in any place except on decayed wood or in water holes. In ravines in the Otis woods where humidity is higher (figs. 1, 2) mosses are a little more common, not growing on the ground, but on sticks, stumps, or bases of trees. These are almost invariably some species of Hypnaceae.

Of the three leading causal factors mentioned for the sand association, water erosion may be substituted for wind erosion and covering. As long as very active denudation continues on a lake bluff or ravine slope, resulting either in a gradual wearing down of the surface or in slumping, mosses have no chance to become established. While evaporation on the bare slope may be excessive, neither that nor competition with other plants is the primary factor. In the later stages, however, these become the two determining conditions. Wherever the arbor vitae and juniper are present we have a repetition of approximately the same conditions as under the pines and juniper on the dunes. The arbor vitae is near its southern limit at Glencoe and does not form a thick cover, and for this reason has less influence as a shade producer than has the pine. On the other hand, the juniper may be just as dense and as effective in producing shade and in retaining moisture as in the former situation.

ULRICH (12) has made a study similar to that by FULLER in the ravines at Glencoe. Three stations were used which correspond roughly to the three elevations on the ravine slope just described, and the results justify the supposition that evaporation is the main cause of such a difference. The station near the top in what would correspond to the *Polytrichum* area showed the highest rate of evaporation; that on the middle of the slope or the region of mixed mesophytic mosses gave a lower rate; that at the bottom or the area of Hypnaceae gave a still lower rate during a part of the season, although at times it was slightly in excess of that midway up the slope. This is exactly what we would expect from the nature of the species present and a comparison of the conditions in other regions where they are found. Competition with other

plants is no doubt an important factor on many such slopes, as they offer conditions increasingly favorable to other ground flora. Erosion decreases in importance as a determining factor in proportion as the mesophytism increases. When the ravine reaches its second denudation period, accompanied by greater sunlight and evaporation, the mesophytic mosses are eliminated along with the other mesophytic undergrowth; but these may reappear when the slope has once more attained a relatively permanent condition, and continue on until the climax association is reached, or may even persist into this association if logs and stumps are present.

In the open oak forests the moisture supply in air and soil probably is again largely the controlling condition, as in the oak-forests on dune sand. Other plants do not occupy the ground to so great an extent as to exclude mosses because of lack of space alone, and there is little probability that the mosses would become shaded to a sufficient degree to shut out the light and prevent the necessary photosynthetic work. Just why there is so great a scarcity of mosses in the more mesophytic oak or oak-hickory forests, as well as in the beech-maple climax, both of which provide relatively high humidity and low evaporation rate (6), has not been fully determined. Competition with other plants may be accountable to a great extent, but even this does not seem sufficient to cause the almost complete elimination of mosses from these forests. In some places there is a continuous succession of dense ground vegetation during most of the growing season, which might be able to prevent the development of mosses; but in other places the vernal flora does not seem to be followed by a conspicuous aestival flora, yet mosses are not present. Perhaps the competition with the vernal flora in its prime, when most mosses attain their greatest growth, may be sufficient to prevent both spore germination and vegetative growth at this time, so that presence or absence of ground vegetation later in the year is of little consequence. The fact that when old logs are present, mosses are common upon them when not found on the ground, would indicate that they had not been able to hold their own against the herbaceous plants. Another factor which may have a

decided influence is that of the chemical change in the soil due to increase of humus. Just what the difference is which seems favorable to the germination of the seedlings of the climax trees and not to those of the former association, and how much of this difference is chemical and how much physical and related to light, are questions for future solution. Whatever it is, it would probably affect mosses as well as other plants. That an acid condition of the substratum alone is not detrimental is indicated by the luxuriant growth of many species on decaying wood and upon needles of conifers.

The great abundance of mosses in the upland oak forests along the Des Plaines River seems to be related to the slightly greater humidity of the atmosphere and larger supply of available soil moisture. There are indications that much of this region has been and still is at certain seasons somewhat swampy, so that there may be some question whether it belongs in the xerarch succession proper or should be placed in the hydrarch swamp series. While the final outcome would be the same in the two series, the intermediate successions would differ to a very large degree. The presence of the relic species in the grazed woods or partially cut-over land seems to be explainable by the fact that they are mosses of wide extremes of habitat, and are highly light tolerant. The change in environment appears to have taken place so gradually that the mosses have been able to become adapted to the greater xerophytism without themselves being materially altered.

The successions on morainic drift may be summed up in a few points. Mosses are entirely absent on the newly eroded bluffs and in the early stages of the ravines. They do not become conspicuous in the ravines until a rather advanced state of mesophytism has been reached, but they probably play an important part in the stabilization of the clay surface and addition of humus, which hasten the advance of the seed plants. Mosses appear in the conifer stage on the bluffs, forming part of the heath mat under the juniper. They are most abundant in the middle aged ravines, before the second xerophytic stage is initiated by the widening of the ravine and decrease of the angle of the slope.

On the oak upland and in most oak and oak-hickory forests of the subclimax type mosses are nearly absent, particularly where decayed logs are not to be found. The same paucity of mosses occurs in the beech-maple climax forests of this region, where competition with other plants or chemical conditions of the soil may be the leading cause. The increase in moss flora along the Des Plaines River at Wheeling seems to be a result of former and present better supply of moisture in soil and atmosphere.

Rock successions.—The rock successions are poorly represented in the Chicago region. The early pioneer stages of lichens and mosses, however, can be distinctly traced at Lemont, Illinois, near the Des Plaines River, on rocks of Niagara limestone which have recently been exposed, on the sides of an old stone quarry, on a cliff in an open pasture, and in several small ravines. The early crustose lichens are followed by *Bryum argenteum* and *Grimmia apocarpa*. *Ceratodon purpureus* seems to succeed these or even to appear with them on the flat rock surfaces, either on the top of the cliffs or on the bowlders. Many rocks have been exposed during recent excavations in straightening the channel of the stream. These are frequently well covered with crustose lichens, and the first moss to invade the lichen zone is *Bryum argenteum*, so that in this case at least this species is a pioneer moss. Elsewhere on rocks it seems often to come in later than *Grimmia*. At the mouth of the ravines, wherever the rocks are still exposed to xerophytic conditions, the struggle is going on between the mosses and lichens. The pioneer mosses usually smother out the crustose lichens, but in turn may be covered up by small species of the foliose lichen group. The mosses here never become very abundant, nor do they occupy large spaces. On the vertical faces there are numerous small cracks and pits in the rock which offer a better hold for typical crevice species, such as *Funaria hygrometrica* and *Gymnostomum rupestre*. Crevice forms are somewhat more abundant in the cracks of a stone wall at Palos Park where the mortar has disintegrated. At the quarry near Thornton, where the horizontal surface of the limestone has been denuded, there are numerous patches of *Funaria hygrometrica* and *Ceratodon purpureus*. Within the limits of Chicago, at Stony Island, although

the rocks have been long exposed, only very depauperate specimens of these same species occur. The later stages of the rock succession are absent. All of these places, with the exception of Stony Island, are surrounded by agricultural lands, and whatever has been the natural fate of this series has been too nearly obliterated by man to allow of its determination. At Stony Island the top of the rock is covered with prairie vegetation. The presence of a few oak trees seems to indicate that without the intervention of man the grasses would have been followed by an oak forest. The conditions at Lemont may have been much the same. In the ravines themselves the mosses belong almost without exception to the Hypnaceae and are without sporophytes, and hence are difficult to determine. *Brachythecium digastrum* is a rather common species.

The Carroll Creek ravine, where humidity is much greater and there is considerable seepage of moisture over the rock surface, is a much more favorable habitat for mosses than are the rock outcrops in the Chicago region. The number of species is not large, but those which do occur are plentiful and they form a thick covering over the rocks. Wherever the stream comes in contact with the rocks, and in other very moist places, liverworts are the first plants. Above the liverwort zone, or on rocks less closely in contact with the water, is the zone of crustose lichens. These are usually followed by foliose lichens, although quite often the pioneer mosses may succeed the crustose and contend for possession with the foliose lichens. The first moss is *Grimmia apocarpa*. On rocks in the open, exposed to strong insolation the greater part of the day, this species is abundant both on horizontal and vertical surfaces. Accompanying this is *Bryum argenteum*, which may occur almost if not quite as early, and in even greater quantity, particularly on horizontal surfaces.

This region offers the best illustration of a very definite succession of mosses on mosses. Here a second or even third moss stage is common and may occur on rocks in the open as well as on those in mesophytic shaded places in the ravine. The species which constitute the later stages differ in the two situations. In sunny places *Bryum argenteum* frequently forms the second stage,

with some Hypnaceae as the third vertical layer. An especially good example of this was found on a low rock situated on a hillside in an open pasture, and at some distance from the stream. The top of the rock sloped a little in the downhill direction and was slightly lower than the ground at the upper edge, but was perhaps 2 feet above the ground at the lower side. Numerous bushes overhung the upper border, but the lower part was exposed to full sunlight. On the shaded vertical face was a small quantity of a liverwort and an extensive growth of crustose lichens. The liverwort did not grow over the edge at the top, but the crustose lichens which had spread over much of the upper surface were being overgrown by foliose lichens. Growing among and over these was *Grimmia apocarpa*. Overlying the edge of the *Grimmia* and in many places entirely covering it was *Bryum argenteum*, forming a thick compact mat over a large part of the remainder of the rock, except at the upper side where soil had washed over the surface from the ground in contact with it above. Here *Brachythecium acuminatum*, growing partly on the soil, was extending out over the *Bryum*, forming a third moss layer. Small patches of lichens and of *Grimmia* here and there indicated that these at one time had been pioneer plants over the entire surface. When the two more mesophytic species came in, they had developed more rapidly on the part of the rock which received the most moisture from the ground and which was also somewhat shaded by overhanging bushes.

In shaded places along the creek in the ravine proper several species of *Anomodon* form the moss stage following the pioneers. As would be expected, the change in species occurs more rapidly in spite of the slope of the rock, which more nearly approaches the perpendicular. In some places the cliffs are quite closely covered with *Juniperus virginiana* and deciduous trees and shrubs. Under these and often overhanging the edge of the cliff is an undergrowth of *Taxus canadensis*, reminding one of the *Juniperus communis* under the pines in the dune region, except for the greater mesophytism which is indicated by the herbaceous flora. On vertical rock faces, well shaded and with water dripping over the surface, a luxuriant mass of *Anomodon viticulosus* is the only common

species. On surfaces with a more gentle slope, where the moisture supply is somewhat less but still plentiful, this species, either alone or with *Anomodon rostratus*, forms the second moss stage. Where exposure to evaporation is greater, *Anomodon rostratus* alone, of the two species, occurs. Under the *Taxus* is a close moss carpet in which *Thuidium delicatulum* forms the third moss layer, and the second species is ordinarily *Anomodon rostratus*, which has smothered the *Grimmia* except at a very few points. Other species which help to make up this moss carpet often several inches thick are *Climacium americanum* and *Rhodobryum roseum*. This seems to be a moist habitat even during very dry periods. Another even better successional series was found on a rock on a more gradual slope, well shaded by deciduous trees of an older ecological association, and well above the level of the stream. This rock projected out a short distance from the bank, leaving a small space between the rock and the ground below. On this protected lower surface *Fissidens cristatus* formed a complete covering and in places extended up over the edge of the rock. Growing over this on the upper surface and reaching down over the edge at some points was a thick mat of *Anomodon rostratus*. Upon the *Anomodon* was a third stratum of *Thuidium delicatulum* and a small quantity of *Entodon cladorrhizans*, in all forming a compact mat of considerable depth. No traces remained of the typical pioneer mosses. The lichens showed occasionally under the *Fissidens*. On the *Anomodon* were patches of a powdery lichen and also of a fruticose species, showing that these may develop on the mesophytic mosses. *Climacium* and *Rhodobryum* again formed a small part of the last moss stage. Growing in this carpet of moss were such plants as *Pilea pumila*, *Geranium maculatum*, small ferns, and tree seedlings, indicating that the next succession is to be that of the vascular plants. Many such examples of the vertical succession of mosses are to be found throughout this ravine.

Such a moss carpet has been described by COOPER (2) for the rock surfaces on Isle Royale, and by BRAUN (1) for the conglomerate rocks near Cincinnati, Ohio.

At the top of the perpendicular cliffs there seems to be no special variation in mosses. Backward from the margin the same

pioneer xerophytic species soon give way to the more mesophytic ones. From the edge there is usually a rather abrupt slope upward for a few rods, which is thickly wooded, in most cases with oaks sparsely sprinkled with red cedar, and here and there a white pine. The undergrowth is decidedly mesophytic, and on the rocks are the same mosses already given for the other moist shady habitats. Immediately beyond the strip of wooded land are cultivated fields.

In comparing the sparse moss flora on rocks of the Chicago region with the very luxuriant display along Carroll Creek, where general climatic conditions must differ only slightly, one at once begins to search for the cause of the variation. While the rock exposures around Chicago are not extensive, they are sufficient to serve as a basis of comparison. The rock in both cases is dolomitic limestone, not differing enough in structure to be an important factor. The only outcrop which is near enough to Lake Michigan to be affected by the greater humidity is that of Stony Island, and that is, if anything, more barren than are the other regions. The cliffs and ravines at Lemont are not close to the stream as are those at Mount Carroll, but are on what was probably the river bluff at some past period when the stream contained much more water than at present, in all probability when the Des Plaines River was the outlet of the old Lake Chicago. Now the cliffs are not near any body of water, and in the ravines are only small streams which are nearly dry a part of the year. The stone quarry at Thornton is being worked by a cement factory, so that the exposure, with the exception of the rocks along the top, is too recent to afford any information. The amount of moisture which could come from the pool of water in the bottom of the quarry cannot be great enough to affect the flora on the horizontal rock surfaces above. The quarry at Lemont has been abandoned for some time, and much of the bottom is overgrown with weeds and grasses. The pools of water in the depressions may add slightly to the humidity of the air in the immediate vicinity; while the vegetation growing up from below and that overhanging from the upper edge of the rock undoubtedly adds to shade and contributes to a lower rate of evaporation. The

rocks near the Des Plaines River, thrown out in straightening the channel, have also been exposed for only a short time. It would seem therefore that the recent exposure in some cases and the distance from bodies of water sufficiently large to locally affect the humidity may be two of the reasons for the poor development of rupicole species. Another probably greater factor, at least for Stony Island and Thornton, is the large amount of dust which accumulates on vegetation, very effectually hindering photosynthetic work. At Stony Island there is much fine coal dust from smokestacks and trains, as well as dust from factories. At Thornton a large quantity of fine white dust thrown off from the cement factory accumulates in a thin layer and forms almost a crust, after light rains, on the foliage of all plants. There is less dust at Lemont, where there is a somewhat better development of mosses, but still much more than along Carroll Creek, which is bordered only by forests and farm lands, and is far from any factories. The later stages of succession on the rock outcropping near Chicago, as stated before, have been greatly interfered with by man. Evidently the change from pioneer conditions is extremely slow, and there is no development of true forest, so that all moss stages beyond the pioneer are so far wanting.

Returning once more to the Carroll Creek ravine, in great contrast to the Chicago region there is a narrow valley flanked by steep rock walls upon which direct sunlight falls for only a short number of hours each day. That this has much to do with the lower evaporation and higher humidity is indicated by the more mesophytic undergrowth and the greater luxuriance of mosses on all undisturbed north facing slopes. Whatever moisture enters the air through evaporation from the stream will be carried away slowly, since such a valley is well protected from winds. Another condition which also points to the moisture from the water as an important factor is that the greater growth of mesophytic mosses is found at places where the stream in its meanderings comes close to the rock wall, either on the north or south side of the ravine, and that the mosses are more luxuriant than in other places with a similar exposure but farther from the water. An additional cause may be found in the length of time in which snow

remains upon these north facing slopes. In places sheltered from the warm spring sunlight the snows melt slowly, and the moisture soaks into the humus instead of running off rapidly, as it must do on such an incline when the snow melts more quickly. It is well known that in general the moss flora becomes more conspicuous as we go north into the cold temperate regions. This condition is comparable to that of the northern habitats where much of the snow disappears under the action of sunlight and not of rains. Since these slopes are exposed to a lower degree of insolation even during the summer, the mosses are never subject to extreme desiccation. This cannot be true of the rock habitats which lie within the Chicago region.

The great economic importance of such a moss covering is demonstrated by the growth of seedlings of higher plants upon the moss mat, which leads to the initiation of the tree associations. Herbaceous plants grow to maturity and produce seed on moss covered rocks, with the roots obtaining nutriment only from the decayed moss material. The slower growing tree seedlings can exist in a like manner for several years, by which time their roots may be able to penetrate through the crevices or between the rocks to the soil below. Mosses are very hygroscopic and quickly absorb water during rains, but give it up slowly. Several days after rains water can be pressed from these mosses even though seepage is not an important factor. In addition to this is the immense value of a moss covering on rock slopes to conserve the water supply and prevent flooding of the adjacent land along the lower course of the streams. The great value of mosses in relation to the conservation of moisture and their effect upon the soil was observed by Oltmanns (8). He says:

> A moss carpet acts as a sponge. A dense low carpet with countless capillary spaces between leaves and rhizoids absorbs capillary and superficial water, but obtains little or none by suction from soil and internal conduction. Consequently living and dead carpets of moss imbibe and evaporate approximately the same amount of water. A carpet of moss does not desiccate the soil . . . . they dry it to a less degree than does other vegetation, and they protect dry easily heated soil from desiccation.

Evans and Nichols (5) also discuss the economic value of mosses in such situations.

The moss successions on rock surfaces may be summarized under two main heads: (1) There are at least four factors which are of special importance in accounting for the better moss development on rocks along Carroll Creek than in the Chicago region: the greater humidity in the former place because of nearness to a stream and lessened exposure; a lower evaporation rate due largely to the fact that the rocks are sheltered from direct rays of the sun for a greater number of hours each day; the slow evaporation of the large quantity of water taken up by the moss mat during the gradual melting of the snow, and consequent lack of desiccation; and the freedom from atmospheric dust, common about any large city, which tends to retard photosynthesis. (2) Mosses are of special value on a rock substratum, as soil formers, to form a habitat for herbaceous plants, to initiate the early tree associations, to conserve water supply and to prevent floods by too rapid run-off, and to add to the aesthetic beauty of the landscape.

River bluff succession.—Another somewhat xerophytic habitat is that of a high river bluff as seen at Thornton, Illinois. In this region Thorn Creek, a comparatively small stream, has cut down much below its former level, resulting in drainage of the adjacent land and a consequent lowering of the water table. The trees along the bluff are deciduous and sufficiently scattered to allow penetration of the sun's rays, even during the summer. Because of grazing there is no shrubby undergrowth. Here are such mosses as *Catharinea undulata*, *Leucobryum glaucum*, *Ceratodon purpureus*, *Funaria hygrometrica*, *Polytrichum commune*, and *Physcomitrium turbinatum*, all of which are quite abundant. All of these, except the last, are found in the neighboring swamp forest. *Catharinea*, which is usually found only in the mesophytic forest, is probably a relic from a previous period of greater mesophytism. *Polytrichum*, while often found in rather dry places, seems usually to originate in mesophytic or even swampy habitats, so that it also is likely a relic. *Leucobryum* and *Funaria* have a wide range of habitat, and may be either relics from a more moist condition, or pioneers on soil constantly becoming more xerophytic at the surface. *Ceratodon* and *Physcomitrium* are doubtless sub-

sequent species, as they are found only in somewhat xerophytic species.

We have, therefore, a retrogressive succession indicated by the moss flora, which is a mixture of relic or antecedent, typically mesophytic species and the subsequent xerophytic forms. Such retrograde successions are not uncommon wherever surface conditions of soil water and exposure to evaporation have undergone rather gradual modification.

Hydrarch successions

Under this heading have been included all successions originating in water or very moist habitats, with the exception of the moist rock succession already described.

Floodplain succession.—This succession was studied at several points along the Des Plaines River, as at River Forest, Riverside, on the east bank at Wheeling, and also along Carroll Creek. The work has been of importance only for its negative value in establishing the fact of almost entire absence of mosses in such associations. Late in the season a few immature plants may sometimes be found, but these seem never to reach maturity if growing on soil, although a few well developed sporophytes may be found on plants growing on logs above the high water level. The true floodplain is subject to inundation during spring rains and during high water at any season. A great quantity of fine alluvial sediment is carried over the land and settles to the bottom with the recession of the water, leaving a crustlike layer of variable thickness over the ground and on any vegetation which may be present. The moisture conditions, except during the flood period, are favorable to spore germination; but the frequent deposit of fine material, particularly at the period when the moss plants would begin the season's growth, seems to be sufficient to destroy the ephemeral protonema which by any chance may begin to develop. The immature plants found later in the season probably come from late germination of spores which have escaped destruction or which have reached the floodplain from the surrounding uplands after the spring inundation.

Evaporation on a floodplain is not excessive, and the available supply of soil moisture is high, so that these two conditions

cannot cause the absence of mosses. Competition with the abundant herbaceous flora either in the spring or summer is only a secondary cause, if worthy of consideration at all. If competition were a prime factor, we should find somewhere in the floodplain succession, either in the horizontal series from the water back to the upland or in the series from the standpoint of time from the floodplain formed by the younger stream as it begins deposition, up to the old floodplain of the mature river which has nearly reached base level, an association in which mosses take an important part. This has not been observed on any of the floodplains under consideration. It is not, therefore, a case of being crowded out by other plants, but rather an inability to survive the unfavorable dynamic conditions along a depositing stream, which are as effective in eliminating mosses as was the active erosion of the earlier stages in the stream's development.

SPRING STREAM SUCCESSION.—At Otis, Indiana, and New Lenox, Illinois, are numerous springs, the water of which is highly impregnated with iron compounds. As the water comes in contact with the oxygen of the air, bog iron ore is produced which builds up mounds about the outlets of the springs until the water can no longer force its way to the top for escape, and finds a lower exit where there is less resistance to be overcome. Very frequently numerous species of plants make up a large part of the foundation structure of the tufa. Taking part in this tufa formation is a coarse moss, *Brachythecium rivulare*. The chemical substances in the water penetrate the plant tissues which, as they grow old, resist decay and form a porous rocklike mass. In the larger stream forming the outlet of such springs at New Lenox are several species of *Amblystegium* growing on submerged sticks and stones, but these do not enter into the tufa formation. A few other species, not typically water forms, grow on sticks which emerge from the water.

A somewhat comparable case of the formation of travertine in the waterfalls of the Arbuckle Mountains in Oklahoma has been described by EMIG (4), in which the two mosses *Didymodon tophaceus* and *Philonotis calcare* are the species involved. Still another species, *Cratoneuron filicinum*, has recently been collected by

Cowles at Turkey Run, Indiana, where it is a common species aiding in the tufa formation in the waters of similar mineral springs (11).

Pond and lake successions.—The pond and lake successions may be classed in two general groups based on the ecological development. The early successions are represented in the Chicago region by two subdivisions, the pine pannes examined at Miller and the lagoons of Buffington and Long Lake, Indiana. The later successions may be found in the swamp forests at Wilhelm and Furnessville, Indiana, and Thornton, Illinois, and the bogs at Mineral Springs and Hillside, Indiana.

Early stages of pond succession.—Pine pannes.—The pine pannes are depressions among the dunes, so low that water which seeps through the sand from the lake, or in this case partly from the Grand Calumet River, reaches the surface or even may rise above it. Some of the depressions may be quite dry during the summer; others may have sufficient water to withstand ordinary summer drought, and remain wet throughout the year. Surrounding the more or less circular body of water in the center of the larger depressions is a border of pines of the same species as previously mentioned for the pine dunes. As a general rule we do not find a typical pond flora even in the center, probably because the quantity of water may be subject to great variation during the year. Sedges and marsh grasses are common, especially near the margin. Only one species of moss forms an extensive growth, namely, *Campylium stellatum*. It may be entirely submerged in the shallow water, but seems to thrive equally well along the edge where it emerges, and, as a relic from a former hydrophytic condition, may even be found on the higher ground at the edge of the tree zone. It is not a floating species in the pannes and is not found in deep water, yet it is the same species which forms much of the substratum of the floating islands in the lagoons at Buffington. While it cannot be considered as a tufa former, it aids materially in filling up such depressions. On the higher land among the trees other mosses are either absent or, if present, are of the same species as already given for the early pine dunes.

Lagoons.—The lagoons at Buffington have been described in the first part of this paper. The water is much deeper than in

the pannes, and the vegetation varies from the submerged species in deep water to the forests on the drier ridges. Floating in the deeper lagoons and sometimes emerging in the more shallow ones is a large quantity of *Drepanocladus fluitans*, *D. aduncus*, and *Campylium chrysophyllum*, and perhaps other closely related species. Around the margin of many lagoons are *C. stellatum*, already mentioned for the pine pannes at Miller, and *Bryum ventricosum*, which has also been found at Long Lake and Pine in much the same situations. In the larger lagoons are several floating islands, of which *C. stellatum* forms a large part of the foundation. In the larger lakes about Chicago, such as Wolf and Calumet lakes, the same marginal soil species of moss occur, but so far none has been found floating or submerged in the deeper water.

Wherever mosses appear, either floating or along the margin of ponds, they aid greatly in the conversion of depressions into land by promoting the advance of other terrestrial plants. There seems to be little difference in the mosses of the pannes and lagoons, except that which can be accounted for by the more shallow water in the former, which may subject the plants to seasonal periods of desiccation, and which would prohibit anything in the way of floating mosses or of floating islands. In both cases it is quite evident that mosses are an important class of plants in the early stages of the pond successions.

Late stages of pond or lake succession.—Swamp forests.—When comparatively shallow ponds and lakes pass from the aquatic conditions, the progress toward the later associations is by growth of vegetation upon the bottom along the margin. Waste material accumulates. In time the open water in the center is entirely eliminated, and a swamp results, which, depending on local conditions, may pass into a prairie where mosses take little part, or into a forest where they may be of prime importance. The Thornton and Furnessville swamps are illustrations of the latter type of development in rather early stages, while that at Wilhelm gives a later condition much more mesophytic. The first two are still characterized by depressions and hummocks, which are rarely encountered in the Wilhelm forest. Although humidity, shade,

and other factors of environment do not differ widely in the three areas, only five moss species have so far been found common to all. These are *Ceratodon purpureus*, *Mnium cuspidatum*, and *Catharinea undulata* on higher land or on logs, and *Brachythecium rutabulum* and *Amblystegium radicale* in low wet places. All except the first are mesophytic species. The *Ceratodon* occurs rarely and then on sticks which are in rather dry locations in the open or along the margin of the swamps. *Sphagnum* and *Leucobryum* are found only at Thornton, the former growing on the ground in depressions, and the latter on hummocks. Wilhelm far surpasses the other forests in the total quantity of the moss flora. *Thuidium delicatulum* grows abundantly on decaying logs and occasionally on the ground, and is perhaps the most conspicuous species with the exception of *Mnium cuspidatum*. *Thuidium recognitum* and *Anomodon rostratus* are found in smaller quantities, usually on logs or tree bases. Several of the very mesophytic species, such as *Climacium americanum* and *Rhodobryum roseum*, are common both on logs and on the ground. The shade is dense, and decaying plant material forms a thick layer on the forest floor. The moss display is of greater luxuriance than elsewhere in the Chicago region and is a close rival of that of the Carroll Creek ravine.

Bog forests.—The two bogs studied within the limits of the region under consideration are the tamarack bog of Mineral Springs and the *Sphagnum* bog near Hillside. Several typical associations in the ecological development can be distinguished: the sedge mat, shown at Mineral Springs; the shrub stage, well developed in both bogs; and the tamarack tree association at Mineral Springs. An additional division might be made of the *Sphagnum* moss association at Hillside, but this is a slightly different line of development rather than another ecological association.

As stated before, the bog successions are distinguished in origin from the pond successions, in that they are formed on sedge mats which grow out over the surface of deep lakes, forming quaking bogs, which may remain in a very unstable condition for many years. The first association to be found at Mineral Springs at the present time is a mixture of bulrushes, cat-tails, and

sedges, all of the early aquatic plants having disappeared. Mosses are about equally conspicuous over the whole of the sedge mat, and consist chiefly of six species, all long-stemmed and of somewhat upright habit of growth. They form a rather close packing about the roots of the other plants. All are very hygroscopic and grow partly submerged. The most noticeable is *Calliergon cordifolium*. The others are *Campylium stellatum*, *C. hispidulum*, *Drepanocladus aduncus*, *D. fluitans*, and *Brachythecium rivulare*.

In the shrub association, where the shade is somewhat increased, these species continue, but decrease in quantity. New species do not seem to come in until the late shrub or early tree associations which again show no distinct line of demarcation, but merge into each other. It is here that we get the first development of *Sphagnum* in the Mineral Springs bog. *S. palustre* occurs usually in low wet depressions and has not formed a very extensive growth either among the shrubs or in the tree association where it becomes more abundant.

COOPER (**2**), in his paper on the mosses of Isle Royale, discusses the presence and absence of *Sphagnum* in bogs. He concludes that *Sphagnum* comes in on the sedge mat following sedges of low growing habits, which produce little shade and offer only slight obstruction to the spread of the moss by vegetative growth. The inference is that *Sphagnum* does not germinate in shade, although it may spread into forests by vegetative growth from outside regions.

This theory does not hold for the swamps and bogs of the Chicago region. In the Mineral Springs bog the most common sedges are relatively large and coarse. At Hillside the early sedge stages are past, but the species still present are all tall and coarse. In the former bog *Sphagnum* does not appear on the sedge mat; in the latter *S. recurvum* has in most places entirely replaced all early associations. At Mineral Springs *S. palustre* begins in the transition shrub-tree area, and becomes most abundant among the tamaracks, where it is frequently found entirely disconnected with any present *Sphagnum* region even in the transition association. There is no evidence that it has spread from a less shaded place of germination on the sedge mat, and there seems to be no explanation of its presence other than that it has been able to

start under the shade of the trees and shrubs. North of the Mineral Springs bog is a low, flat, sandy plain covered with shrubs and marsh grass. The undergrowth is a compact mass of *Sphagnum*. In many old lagoons which have reached the shrub stage or which have a rank growth of swamp grasses, *Sphagnum* is growing in rather dense shade, but whether it originated in shade or sunlight cannot now be determined. Another case which is similar to that of Mineral Springs is the presence of *S. subsecundum* in isolated patches in the depressions of the Thornton swamp. There is no connection whatever with outside *Sphagnum* areas. In fact, no *Sphagnum* has thus far been discovered in the open regions around the swamp. Many of these patches are in the interior of the forest, and all are well shaded during the summer. It is quite true that in both the Mineral Springs bog and the Thornton swamp the trees are bare of foliage during the winter season, and therefore sunlight will reach the ground during the early spring. This argument, however, can be applied equally to the sedge association, where there is little shade from the coarse sedges until the new growth has begun. In this region, therefore, it appears that *Sphagnum* must be able to germinate under shade, and that it may be present in forests without having reached these habitats by vegetative encroachment from outside areas. This conclusion is borne out by work done upon the germination of *Sphagnum* by GEORGE L. BRYAN. The results of the study have not yet been published, and I am indebted to the kindness of W. J. G. LAND of the Botanical Department of the University of Chicago, under whose direction the work was carried on, for permission to refer to the results. BRYAN made many careful experiments upon the germination of *Sphagnum* spores under various conditions of soils and sunlight, and found that germination occurred in all degrees of sunlight and in darkness itself. Apparently there is some other determining factor which controls the presence of this group of mosses.

The tamaracks form a border about the bog. On the outer margin they are being displaced by other bog trees, as *Betula lutea* and *Nyssa sylvatica*. The tamaracks grow on hummocks, while the depressions between them may be very wet or even filled with

standing water. A large number of species of moss which have not been found in the previous bog associations occur here, on the ground, on sticks, or on logs. *Calliergon cordifolium*, the two species of *Campylium*, the *Brachythecium*, and *Drepanocladus aduncus* continue, often on partly submerged sticks. In slightly higher situations, but on ground that is still very wet, are *Leucobryum glaucum*, *Climacium americanum*, and *Thuidium delicatulum*. With the exception of *Leucobryum*, these species are also found on logs and sticks. *Anomodon rostratus* comes in where there is less moisture, particularly about tree bases. Here, as in the other mesophytic moss habitats, the soft hygroscopic mass of moss tissue forms a favorable place for the germination of tree seedlings and the seeds of other plants. As one approaches the higher land adjoining the sand dune to the north, the moss growth becomes less in quantity, but does not change very much in species until the dune itself is reached.

In the Hillside bog, a large part of which has reached the shrub stage, but in which there is much less water than at Mineral Springs, *Sphagnum recurvum* has been, and in places still is, the dominant vegetation. It must have reached a very luxuriant development in the recent past, but is now on the decline. In many places *Aulacomnium palustre* forms a second moss stage growing on *Sphagnum*, and this is frequently accompanied by *Polytrichum commune*. COOPER describes such an association in the *Sphagnum* bogs on Isle Royale. The bog itself has not yet developed the tree association, although with respect to moisture conditions it has advanced much beyond the bog at Mineral Springs. It is surrounded by climax beech-maple forest, and it is quite likely that this will be the fate of the bog if left to nature's influence. In the adjoining beech-maple forest *Catharinea undulata* is again the only moss of any prominence.

Table II represents the hydrarch succession from open water of lagoons and ponds to the climax forest. Once more the great importance of pioneer mosses in the advancement of the higher plant associations is shown. The economic value of shallow ponds is slight; while on the other hand they may be very injurious in that they harbor larvae of insects, harmful to man, so that the

elimination of such swampy regions may be very desirable. By the filling up of depressions the area may be made productive either as prairie or forest. The poorly drained deeper ponds are probably as little to be desired from an economic standpoint, since the water will not support the life of aquatic animals of commercial value. Consequently any natural agency which will further the change from hydrophytic to mesophytic conditions will add to the number of acres of productive land reclaimed from a state of total non-productivity, and also lead to better health conditions for the inhabitants of the surrounding country.

TABLE II

PRESENCE OF MOSS SPECIES IN ASSOCIATIONS OF HYDRARCH SUCCESSION

Species	Open water	Sedge mat	Tamaracks	Swamp forest	Beech-maple
Amblystegium riparium..	P	.....	P	.....	.....
Anomodon rostratus.....	.....	.....	P	P	.....
Aulacomnium palustre...	.....	P	.....	P	.....
Brachythecium sirulare..	.....	P	P	P	.....
Campylium stellatum....	P	P	P	.....	.....
Campylium hispidulum..	.....	P	P	.....	.....
Calliergon cordifolium...	.....	P	P	P	.....
Climacium americanum..	.....	.....	P	P	.....
Catharinea undulata....	.....	.....	.....	P	P
Drepanocladus aduncus..	P	P	P	.....	.....
Drepanocladus fluitans...	P	P	.....	.....	.....
Dicranum scoparium....	.....	.....	P	P	.....
Entodon cladorrhizans...	.....	.....	P	P	.....
Leucobryum glaucum....	.....	.....	P	P	.....
Mnium cuspidatum.....	.....	.....	P	P	.....
Polytrichum commune...	.....	.....	P	P	.....
Rhodobryum roseum....	.....	.....	P	P	.....
Stereodon haldanianum..	.....	.....	P	P	.....
Thuidium delicatulum...	.....	.....	P	P	.....
Thuidium recognitum...	.....	.....	P	P	.....

The pannes about Miller are mostly of recent origin and are not within easy reach of other habitats of aquatic mosses. This may account for the fact that the few species are present. The mosses found growing in all of these ponds, so far as observed, propagate vegetatively only, or with very rare spore production, thus virtually prohibiting their spread into distant ponds except when carried by birds or other animals. As previously mentioned, these mosses must be able to make a good recovery after periods of desiccation, and must also be able to resist covering to some extent, as these pannes

are subject to occasional dry seasons and frequent deposit of sand. The presence of the mosses soon leads to accumulation of humus over the sandy bottom and initiates the growth of semihydrophytes.

In the lagoon region is a far more extensive pond area, both as to actual number of ponds and variation in ecological development, caused by depth and size as well as by age of the individual lagoons. The chance for transfer of mosses from one pond to another is much better; the variation in depth permits the growth both of floating and fixed species, while the greater age has allowed time for accumulation of more humus, which leads to the introduction of still other species, as well as perhaps a more luxuriant growth of all. With these conditions comes the rapid advance of the shrub and forest or prairie successions. In the swamp forest the moss flora becomes increasingly a dominant factor in humus accumulation as the ecological succession advances toward the climax, but begins to decline with the close approach of the beech-maple association. This appears to be a result both of competition with other ground flora and of the smaller supply of available water near the surface.

Very little work has been done in determining conditions for plant life in the bogs, but from the xerophytic structures of many bog plants, and the shallow root systems of the trees, COWLES concludes that, while moisture is plentiful, the chemical content of the water is such as to have a toxic effect upon the root development of plants, and to prevent absorption of water to a great extent. In other words, this is a physiologically xerophytic habitat for seed plants. It is not known how far this may influence the development of mosses; that it is not very injurious is proved by the great abundance of some species, such as *Sphagnum*. On the sedge mat the shade may be considerable when cat-tails are abundant, but the sun's rays reach the ground more directly than in the forest. The humidity near the ground is probably greater than among the trees, but evaporation at times is also much greater. The mosses occupy the small spaces around the roots of the fen plants and often cling closely to them, forming a packing between the stems, but there are no large masses. In some places there is a luxuriant growth of marsh forget-me-not and other species of

low growing seed plants which nearly smother out the mosses. The increase in shade and possibly other conditions in the late shrub stage and early tree association apparently are unfavorable for most of the old herbaceous species, and new ones have not taken their places, so that there are large areas unoccupied by such ground vegetation. As in the pine dune, so also here we may have toxicity produced by decay of conifer needles. This probably does not greatly retard the moss development, although it may account in part for the change in species. With herbaceous plants, on the other hand, it may result in almost total elimination. The rapid increase of quantity and number of species of moss in the early tree association, therefore, is directly related to these environmental conditions. The greater shade and lower temperature are both more favorable to moss growth, and added to these is the lack of competition with other plants.

As the tamaracks are replaced by deciduous trees, the mosses give place to herbaceous seed plants. The chemical condition of the subsoil changes, more humus accumulates, moisture and humidity decrease. The mosses now are crowded out of their former locations until, with few exceptions, they persist only on sticks, logs, and tree bases, and we find in their place many ferns and seed plants. Competition seems to be the great cause of the elimination. Some general conclusions regarding the pond and lake successions of mosses are as follows.

Very few mosses appear in the pannes, but those which are present are coarse and aid in filling up the depressions. The lagoons are favorable habitats for floating species, while other mosses are abundant along the margin. Both produce material which is added to the muck on the bottom and which provides nourishment for other plants. Still other species assist in the formation of floating islands. In the bogs a few species of semi-aquatic mosses appear in the early fen stage in considerable quantities. There is a slight decrease in quantity in the shrub stage. A marked increase in quantity and number of species is evident in the early tamarack association and continues until the tamaracks are replaced by deciduous trees, making the tamarack the dominant moss association. In the later deciduous association there is

a continuous decline in the moss flora until the climax beech-maple forest is reached. Competition with other plants seems to be the determining factor as the successions advance beyond the semi-hydrophytic.

Conclusions

1. In the successions on sand, mosses are most abundant, both in number of species and in total quantity in the stage; in which they first become very noticeable, the pine stage; and they decrease through the early oak stages to either the oak or the beech-maple climax.

2. In the swamp and bog successions the greatest dominance of mosses is found usually in the swamp or bog forest association, which may or may not directly precede the climax.

3. The mosses found in running spring water and in stagnant water are of different species, but nearly all belong to the same family, the Hypnaceae.

4. The succession on floodplains is unimportant because of constant deposit of sediment over the germinating mosses.

5. Mosses are among the highly important pioneer plants on bare rock surfaces, and continue abundant far into the forest association.

6. From an economic standpoint mosses are of the greatest value in several respects. They are soil formers and provide favorable habitats for germination of higher plants. They assist largely in forming the surface mat over deep lakes and in filling up shallow bodies of water. They may take part in building up rocklike substances, as tufa. They help to make up floating islands on which higher plants may grow. They conserve moisture, and give it up slowly, thus aiding in the prevention of disastrous floods in the surrounding regions. They prevent erosion of clay or sand surfaces.

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OVULIFEROUS STRUCTUUES OF TAXUS CANADENSIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 267

(WITH PLATE XXIII AND SIXTY FIGURES)

A. W. DUPLER

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Introduction

Following a recent paper (13) in which the writer gave a description of the staminate structures of *Taxus canadensis* Marsh., this paper deals with the ovuliferous structures, namely, the primary shoot, the secondary shoot, and the ovule, describing both the development and vascular features, together with a discussion of the morphological questions raised by these structures. The purpose in this investigation was twofold: (1) to compare *T. canadensis* with the European *T. baccata*, and (2) to look for new evidence bearing on the morphological problems of these structures in the genus. While no pretense of finality is made in this connection, it is thought that some additional evidence has been secured bearing on these problems. Since the female gametophyte has already been described (12), only such reference is made to it as may be necessary. For a statement as to materials and methods, the reader is referred to previous papers (12, 13).

Historical

Taxus has engaged the interest of botanists for a long time, the ovulate features, the gametophytes, and the early embryogeny especially receiving attention. The literature dealing with the ovulate structures is quite extensive, much of it being found in connection with descriptions and discussions of other conifers, and is based almost entirely upon *T. baccata*, very little dealing specifically with *T. canadensis*. The two forms are similar (6), and much which has been written will apply equally well to both forms. It would be impracticable to include a complete summary of all that has been published on the subject, a general summary sufficing,

more complete references being available in the accounts of STRASBURGER (35), RADAIS (24), and WORSDELL (39).

The earlier work was based largely on external features, and attempted to homologize the structures with those of the angiosperm flower. This attempt seemingly persisted much later with *Taxus* than with most other conifers, the gymnospermy of *Taxus* not being quite so soon recognized as in other forms. The bulk of the literature deals with the more theoretical questions, the actual descriptive work not being so extensive. The discussion of the literature will be presented in the text of the paper, in connection with the several topics, in this way avoiding repetition and presenting each topic in more complete form.

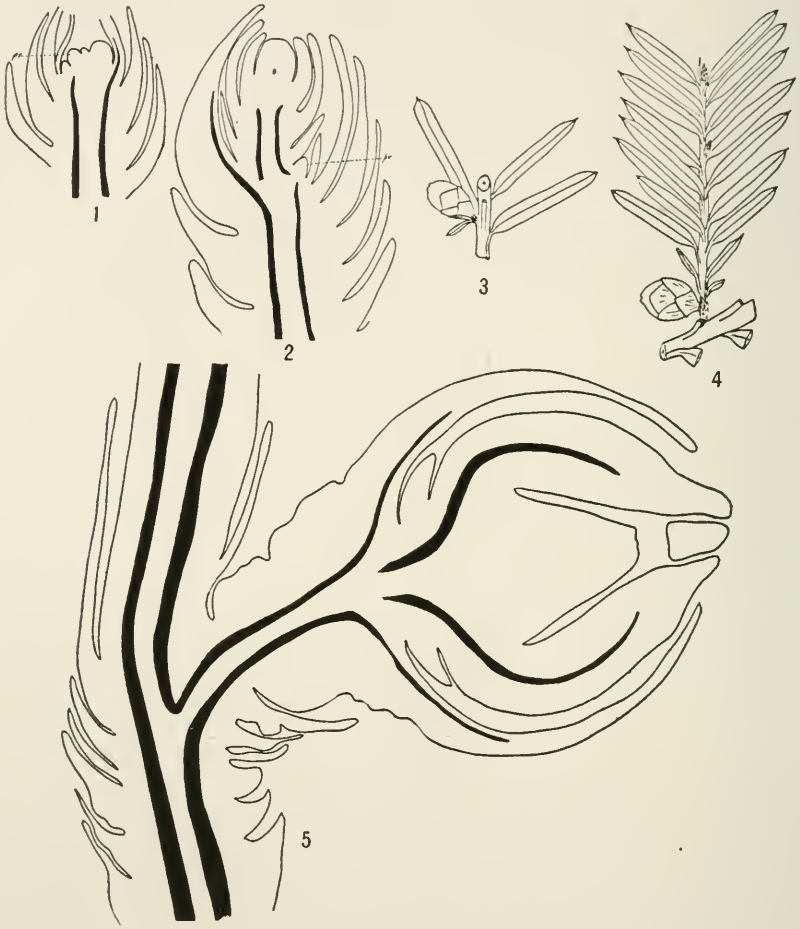
Ovuliferous bud

As previously pointed out (13), three types of buds are formed in the axils of the leaves of a current season's shoot, namely, vegetative, staminate, and ovuliferous. The differentiation of the last is first recognized by the appearance of the rudiment of a secondary axis in the axil of one of its uppermost scales (fig. 1), this rudiment appearing early in July. The ovuliferous bud begins early in the spring, as a conical rudiment in the axil of a young leaf, shortly after the beginning of the growth of the vegetative shoot, forming usually nearer the tip than the staminate buds. STRASBURGER (36) found the first differentiation of the ovuliferous bud in *T. baccata* to occur about August 1. The structure can be distinguished by external features with certainty only when the ovule has reached such size as to protrude beyond the scales, usually not until spring. JÄGER (15) says that the ovuliferous bud of *T. baccata* is evident about February 1, being slightly yellowish, and the vegetative bud being reddish brown; but this is hardly a safe criterion, owing to color variations.

Primary shoot

GENERAL FEATURES.—The ovuliferous organ in *Taxus* consists of two structures: the primary ovuliferous branch, or, as it is more generally known, the primary shoot; and the secondary shoot on which the ovule is borne. The primary shoot arises directly

in the axil of the leaf, and, as STRASBURGER (35) pointed out for *T. baccata*, begins with two transverse scales, following which are a number of scales in spiral order, in the axil of one (or two) of



FIGS. 1–5.—Fig. 1, long section of primary shoot showing rudiment of secondary shoot; fig. 2, secondary shoot with young ovule and primary axis tip pushed to side; fig. 3, primary shoot which has developed two small leaves, shown below ovule; fig. 4, primary shoot which has become functionally vegetative, showing ovule at base; fig. 5, median longitudinal section of primary shoot, secondary shoot, and ovule, such as fig. 4; figs. 1, 2, ×24; fig. 5, ×17.

which the secondary shoot (or shoots) arise. The scales of the primary shoot are very similar to the scales of the staminate

strobilus already described (13), having very thick epidermal walls, especially on the outer surface, stomata on the inner surface, and rather large air spaces. They are brownish and lack chlorophyll.

During its first season the primary shoot is a dwarf branch of limited growth, and the development of the secondary shoot results in its tip becoming pushed aside (fig. 2) and remaining dormant for a time. Externally this gives the appearance of a single structure with a terminal ovule, a situation which may explain some of the earlier views as to the position of the ovule. Van Tieghem (37) apparently was the first to point out this behavior of the primary axis. According to Schumann (31), and also Pilger (23), the primary axis ends blindly, and the so-called tip of the primary shoot is the knob of a reduced side shoot which may at times grow out to form a second secondary shoot. When this occurs the primary axis may form a short knob between the two secondary shoots. This view does not agree with the facts and has received but little support.

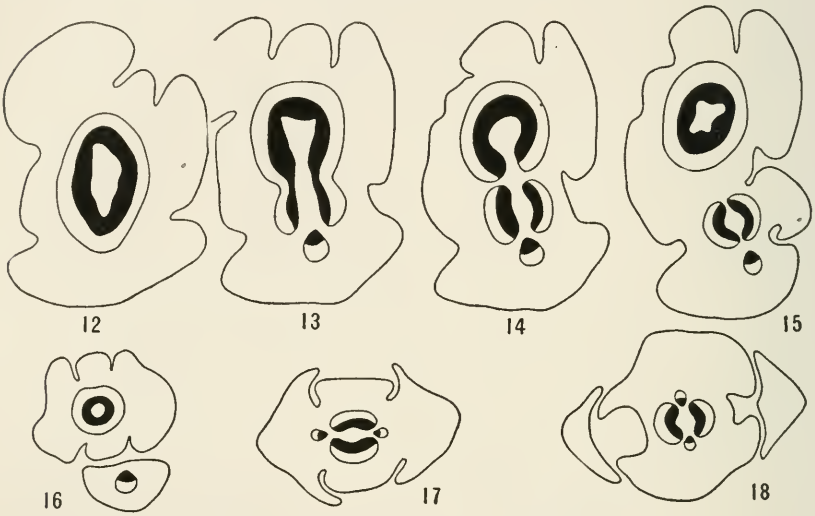
Second season's growth.—The tip of the primary shoot remains dormant until the next spring, when its growth is renewed, resulting either in its continuation as a dwarf structure, as in the first season, or in its growth as a leafy shoot, like that from the ordinary vegetative bud, a fact first noted for *T. baccata* by Strasburger (35). This leafy shoot may bear only a few small leaves (fig. 3) and develop no further during the second season, the subsequent behavior of such small shoots not being known. It also may develop as an ordinary leafy branch, differing in no way from other leafy branches except in bearing the secondary shoot at its base (figs. 4, 5), and, like any other vegetative branch, bearing vegetative and reproductive buds of the next season. Occasionally the primary axis remains dormant as a vegetative bud for a season or more. In such cases the reproductive nature of the first season can be told only by the scars of the old secondary shoot (fig. 6). Normally, however, the primary shoot continues its dwarf and reproductive character for the second and later seasons, producing a few scales as in the preceding season, with one or two new secondary shoots on the new growth. It has been generally assumed that the primary shoot produces fruiting structures for only one

season, and that the maturity of the secondary shoot with the ovule results in the death of the primary shoot as well. This is not the normal situation, as usually only the secondary shoot with the ovule drops from the primary shoot, which remains in the axil of the leaf, a branch scar showing the place of detachment at the secondary shoot from the primary shoot (fig. 6). Detachment of the secondary shoot is probably accomplished normally by the formation of an abscis layer across the base of the shoot. The region of abscission is marked by a narrow layer of platelike cells, rich in protoplasm, outside of which is a layer (5–6 cells wide) of cork tissue, and whose outer border consists of radially elongated cells which form a conical cap to the scar (fig. 7). When collections of *T. canadensis* for this study were first begun, in the autumn of 1913, it was noticed that ovulate buds were to be found on older as well as on the current season's growth, as has since been pointed out for *T. baccata* by Miss AASE (1). This is not due to dormancy of buds which had failed in development, as might usually be assumed, but to the persistence of the primary shoot year after year, producing one or two new secondary shoots each season. This renewal of growth is contemporaneous with that of the primary shoots of new branches, beginning early in the spring, although not becoming recognizable externally until later in the summer, when it can be distinguished by the slight projection which appears at the base of the secondary shoot (fig. 8). Growth is slow, and by the middle of July is arrested, as in previous seasons, by the growth of the new secondary shoot (fig. 9). As these observations show, the primary shoot is a persistent structure and may produce secondary shoots season after season, or become a leafy shoot, the situation being evidence against regarding the primary shoot with its secondary shoot as representing a compound strobilus.

TERMINAL PRIMARY SHOOT.—Several cases were found in which the primary shoot was a terminal structure of the leafy branch (figs. 10, 11), the terminal bud having developed as a primary ovuliferous structure, bearing a secondary shoot. That this may continue to function as a primary shoot for more than one season is shown by the presence of a secondary branch scar a little

FIGS. 6–11.—Fig. 6, long section of primary shoot showing scars of secondary shoots of two previous seasons; primary axis remaining dormant, not producing secondary shoot the season collected; ×24; fig. 7, detail section through scar (note shaded abscission layer and corklike wound tissue external to it); ×140; fig. 8, primary shoot with mature ovule and projection at base of ovule showing external appearance of a normal second season's growth of primary shoot; fig. 9, longitudinal section of primary shoot showing half-grown ovule of current season and young ovule of next season (primary axis tip shown below younger ovule); ×17; fig. 10, terminal primary shoot; fig. 11, longitudinal section of terminal primary shoot (leaf base shown at lower end of figure; note branch scar, left by secondary shoot of preceding season, and that primary axis tip has begun growth for third successive season); aril shown at base of ovule; ×17.

distance below the tip of the primary axis (fig. 11), in which the tip of the primary axis has also begun its renewal of growth for the third successive season. No case was found in which it was known that a terminal primary shoot later became functionally vegetative; but in view of the occasional behavior of the primary shoot as a leafy shoot, it is very possible that a terminal primary shoot may again become vegetative in function.

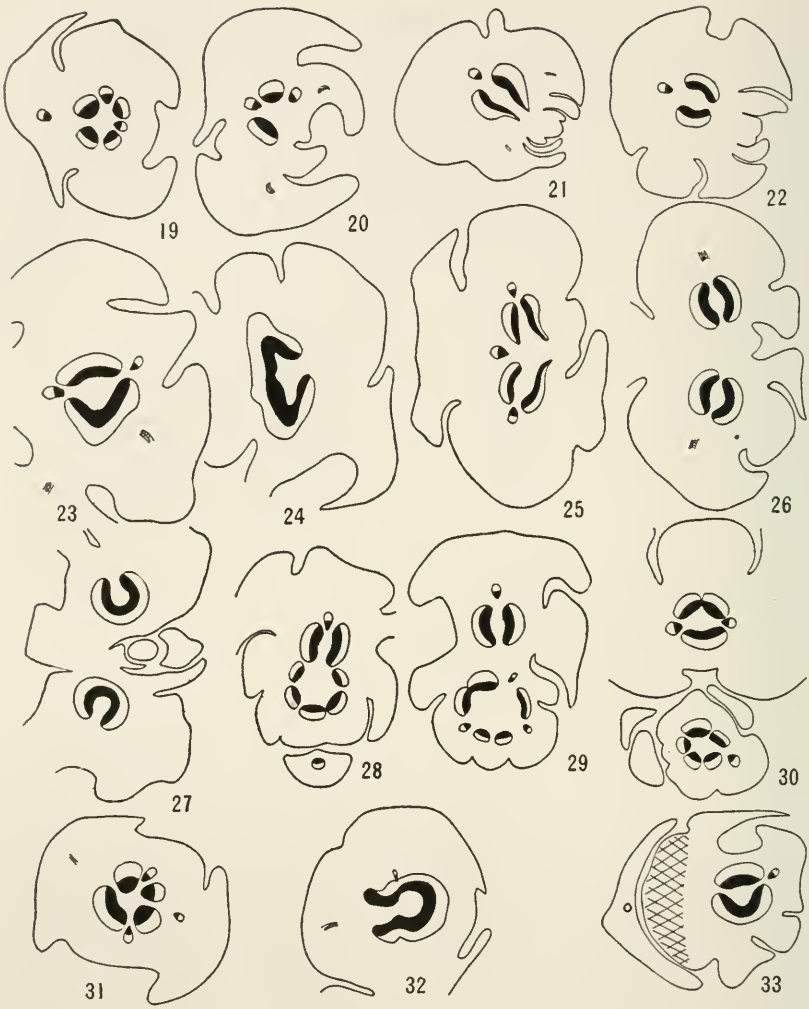


FIGS. 12–18.—Series at different levels showing vascular supply from leafy shoot to primary shoot: fig. 12, vascular cylinder of leafy shoot; fig. 13, trace to fertile leaf and formation of vascular strands to primary shoot; fig. 14, vascular strands for primary shoot separated from main cylinder, showing branch gap; fig. 15, main axis cylinder closed with primary axis cylinder and bundle of fertile leaf farther removed; fig. 16, primary axis cylinder closed; figs. 17, 18, bundles to lower scales of primary shoot, first pair being normally transverse, as shown, remainder usually spiral with occasionally a second transverse pair, as in fig. 18; ×24.

VASCULAR FEATURES.—STRASBURGER (35) was the first to describe the vascular supply of the primary shoot of *T. baccata*, and it is essentially the same in *T. canadensis*. The primary shoot receives two bundles from the axis of the leafy shoot (figs. 12–15). These bundles meet at their edges (fig. 16) and form a complete vascular cylinder, which then gives off traces to the lateral scales (figs. 17–20). At the level of the fertile scale the cylinder organizes

into two large bundles, which pass into the axis of the secondary shoot (figs. 19–22), only a very weak vascular supply passing into the arrested primary axis tip. If there are two secondary shoots, each receives a pair of vascular bundles (figs. 23–27). Should the primary axis grow out into a leafy shoot the next season, a normal vascular cylinder develops, and the vascular supply to the secondary shoot has the usual features of an axillary structure (figs. 28–30). The normal continuation of the primary shoot in its dwarf character during the next season results in a vascular supply to the new growth, similar to that of the preceding season. The vascular tissue of the new growth develops in connection with the bases of the bundles which passed to the secondary shoot of the preceding season, so that a series of sections shows a continuous vascular strand throughout the entire secondary shoot axis, broken by the small scale traces and by a wide gap at the level of the secondary shoot scar, where the bundle supply to the secondary shoot had passed off from the main axis. This gap, however, does not have the ordinary features of a branch gap, being really the leaf gap of the fertile scale subtending it, the bundle supply of the detached secondary shoot being in lateral connection with the main axis at all points, and not separated from it as in ordinary branch gaps (fig. 32; cf. figs. 13, 14). The previously arrested and rudimentary condition of the axis tip accounts for this behavior. The xylem portion of the cylinder is relatively narrow, growth being slow and uniform. Shoots more than one year old do not usually show any growth ring excepting in the region of the secondary branches of the preceding seasons, where the limit between the xylem of the first and second season's growth is very distinct. The xylem is endarch in the cylinder, but in the scales centripetal wood may appear, although the scale traces in general are quite short, frequently ending in the base of the scale.

Morphological nature.—The morphological nature of the primary shoot has been the subject of some question. It seems clear that in *Taxus* the primary shoot is to be regarded as a vegetative shoot of limited growth, persistent for an indefinite period, producing secondary fruiting shoots season after season, as a dwarf shoot functioning only in this way. It may become a vegetative



FIGS. 19–33.—Figs. 19–22, series showing bundle supply from primary shoot to secondary shoot, also transition from normal primary cylinder (fig. 19) to organization into two bundles supplying secondary shoot (fig. 21); figs. 23–27, series showing vascular supply to two secondary shoots on primary axis; figs. 28–30, series showing vascular supply when primary shoot becomes functionally vegetative second season; two large bundles of figs. 28 and 29 belong to secondary shoot, circle of small bundles to primary shoot; figs. 31–33, series through primary shoot at least two years old, showing: fig. 31, usual primary shoot cylinder; fig. 32, large gap formed by bundle supply to secondary shoot (note that bundle supply to secondary shoot is laterally continuous with primary axis cylinder and has not formed branch gap as for normal axillary structure); fig. 33, through branch scar (with crossed lines), and fertile scale; ×24.

shoot of unlimited growth, however, then having both the vegetative and reproductive possibilities of any other branch. The occasional behavior of the terminal bud in becoming a dwarf primary shoot recalls a similar behavior in *Ginkgo*, although one must not infer too much as to relationship on this account.

Secondary shoot

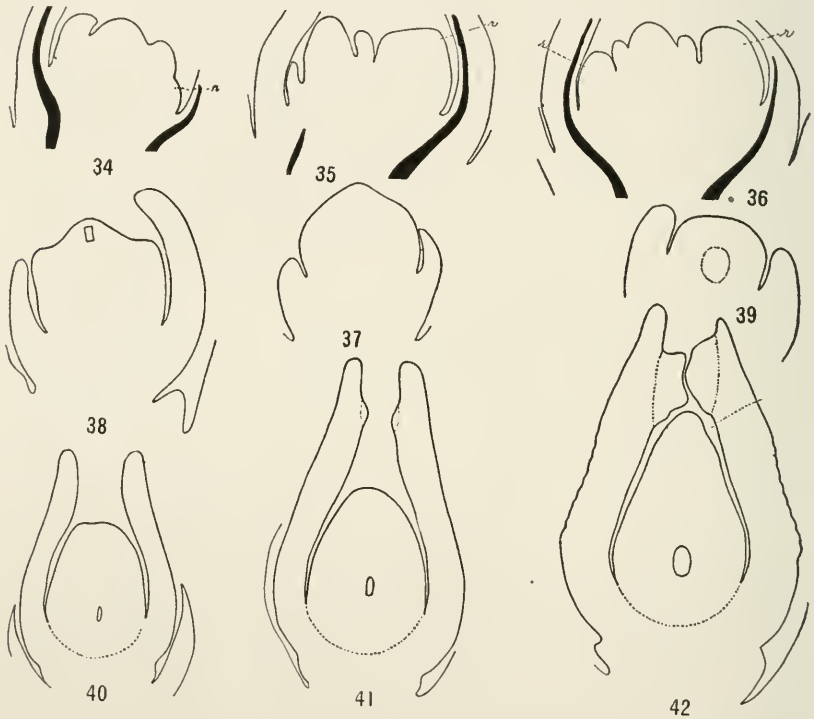
General features.—The primordium of the secondary shoot first appears as a lateral structure in the axil of one of the uppermost scales of the primary shoot (fig. 34), soon becoming conical (fig. 35). It is generally stated that the terminal scale is the fertile one, but one or more small scales usually appear above the fertile one, as was pointed out in *T. baccata* by Van Tieghem (37). Different writers have assigned definite scales of the primary shoot as the fertile one in *T. baccata*, Van Tieghem claiming the eleventh, Strasburger (36) the eighth or thirteenth, and Pilger (23) the seventh; but this varies and is of no special importance. Frequently two of the scales are fertile and two secondary shoots occur, the tip of the primary shoot then appearing between them (fig. 36). In *Torreya* there are usually two secondary shoots on a primary shoot, but Strasburger's account that in rare cases in *Torreya* the primary shoot behaves as a secondary shoot, and bears a third ovule above the two secondary shoots, does not apply to *Taxus*.

The rudiment of the secondary shoot develops rapidly, producing the three pairs of decussate scales in rapid succession, the cyclic arrangement of which is in contrast with the spiral arrangement of the scales of the primary shoot. The first pair stands transversely to the fertile scale. Van Tieghem held that while the scales are decussate there is an indication of a spiral tendency, a view necessary to his theory that the ovule is an axillary structure of the sixth scale of the secondary axis. Practically all investigators agree as to the decussate nature of the scales, as there seems to be no basis for regarding the scales as having a spiral arrangement. The scales of the secondary shoot are considerably larger than those of the primary shoot, and contain chlorophyll, the outer epidermis being heavily cutinized, and stomata occurring on the inner surface. In the early stages these scales protect the young

ovule, but shortly before pollination the tip of the ovule protrudes from between the scales, and with its development they become relatively less conspicuous.

Ovule

Historical.—The ovule of *Taxus* has been the subject of considerable discussion among botanists. The earlier taxonomists, such as Linnaeus (17) and Jussieu (16), regarded the ovule of



Figs. 34–42.—Fig. 34, long section of primary shoot showing lateral axillary rudiment (*r*) of secondary shoot; fig. 35, older stage, rudiment become conical; fig. 36, rudiments of two secondary shoots, primary axis tip between; fig. 37, axis tip of secondary shoot showing bulge indicating beginning of integumentary zone; fig. 38, older stage showing integumentary zone more distinct and differentiation of archesporium (for detail see fig. 61); fig. 39, older stage showing young integument and position of sporogenous tissue (inclosed by dotted line); fig. 40, young ovule about time of pollination, showing barrel-shaped integument and large open micropyle; figs. 41, 42, older ovules and closure of micropyle by plug tissue (for details see figs. 66, 67); figs. 34–39, ×80; figs. 40–42, ×36.

all conifers as a pistil. TREW's observations, in 1767, that the ovule of conifers receives the pollen directly, the representation of TREW's observations by TARGIONI-TOZETTI in 1810 (RADAIS 24), and BROWN's (6) announcement of gymnospermy introduced a fertile topic for debate. For a time these newer views met strong opposition, RICHARD (25), for instance, declaring that there are no plants with naked ovules or without an ovary, and holding that the ovular integument was the perianth and the nucellus the pistil of the flower. BAILLON (2) was also a vigorous opponent, holding the ovule to be a 2-carpel ovary with a single orthotropous ovule. PARLATORE (22), SPERK (34), with others, and even STRASBURGER (35) for a time also held to the ovarian theory of the ovule. Another group, among whom were SCHLEIDEN (29), A. BRAUN (5), SACHS (26), and others, accepted BROWN's view as to gymnospermy. STRASBURGER later accepted the same interpretation, and the question of the gymnospermy of *Taxus* has been generally accepted.

The morphological position of the ovule has not been so definitely settled, and it may yet be regarded as an open question whether it is a lateral structure, foliar in origin and only secondarily terminal, or a true terminal structure, unrelated to the scales in its origin. The first of these views depends upon the assumption that the ovule in gymnosperms must always be related to sporophylls, present or suppressed; the second that the ovule may arise from the axis itself, independent of lateral organs. Among the early workers SCHLEIDEN (30), SCHACHT (28), and others regarded the ovule as terminal to the branch. On the other hand, DON (11), CASPARY (7), and others held to the foliar origin of the ovule. VAN TIEGHEM (37), using the anatomical method as a basis of interpretation, concluded from the orientation of the bundles that the ovule represents the first and only leaf of a shoot of the third order in the axil of the sixth bract of the secondary shoot, a view also accepted by STRASBURGER (35). SACHS (26) regarded the ovule as secondarily terminal, the bract nearest the ovule playing the rôle of the carpel; but later (24) changed his opinion, admitting the ovule to be terminal and a modified stem. STRASBURGER also abandoned his earlier position and held that the ovule is strictly terminal on the axis tip, that no relation to the last pair of scales

can be found, and that there is no ground for VAN TIEGHEM's view. MAGNUS (18), pointing out the cauline origin of the ovule in *Naias*, spoke of it being similar to the situation in *Taxus*, in which he regarded the ovule as terminal. Later workers have more generally accepted the terminal nature of the structure. CELAKOVSKY (8) held that the sporangium is terminal to the axis. WORSDELL (38) accepted and championed this view, stating that "anatomy points clearly to the fact that no axial foliar appendage of any kind exists upon which the sporangium is inserted, the cylinder of the axis being directly continuous into the base of the sporangium." JÄGER (15) speaks of the nucellus in *T. baccata* being formed by the vegetative tip of the secondary shoot. Miss AASE (1), in a recent study of this problem, points out that the vascular supply to the ovule is "contrary to what should be expected" for an axillary structure. She also suggests the possibility of a fusion of sporophylls to form a single structure.

For a solution of the problem two groups of facts can be used directly, the origin and development of the ovule, and its vascular supply; the latter will be treated in connection with the vascular features of the secondary shoot as a whole. There are no known abnormalities with which one can compare the normal situation. *Torreya* apparently presents a similar situation, and thus gives no additional line of evidence.

ORIGIN OF OVULE.—The first indication of the ovular nature of the end of the shoot is the beginning of the integument as a ring around the tip of the axis (figs. 37, 38), and the axis tip itself becoming the nucellus, as claimed by both STRASBURGER (36) and JÄGER (15) for *T. baccata*. There is nothing in the position of the ovule to indicate that it is a lateral structure, and so far as its ontogenetic origin gives a clue one must conclude that the ovule is strictly terminal, cauline in origin, and unrelated to any of the scales. If the scales represent sterile sporophylls phylogenetically, as is most probable, their sporophyll character has been completely abandoned and the axis itself becomes the sporangium, as in some of the angiosperms, where cauline ovules are not uncommon. That the vascular features sustain this view will be indicated later.

MEGASPORANGIUM.—In *T. baccata* STRASBURGER (**36**) pointed out the hypodermal origin of the archesporium, describing it also for *Larix europea*. In *T. canadensis* the sporogenous tissue is also hypodermal in origin, the archesporium becoming differentiated very early in the development of the nucellus while it is yet cone-shaped and the integumentary zone in a rudimentary condition (figs. 38, 61). It may consist of a single cell or a small plate of cells. The periclinal division of the archesporium results in the primary wall cell and the primary sporogenous cell (fig. 62). The wall cell, together with other adjacent cells of the nucellus, divides repeatedly by periclinal divisions, building up a considerable mass of tissue between the sporogenous tissue and the epidermis, the cells of this tissue being in radial rows, at the inner ends of which are the sporogenous cells (figs. 63–65). Morphologically this is the outer portion of the many-layered wall of the megasporangium, and together with the epidermis constitutes the upper portion of the nucellus. The later development results in a considerable mass of sporogenous tissue (fig. 64), out of which one or more cells function as megaspore mother cells (fig. 65), as pointed out in my previous paper (**12**). While I have no preparations showing divisions of the primary sporogenous cells, the amount of sporogenous tissue present indicates that this takes place, contrasting with the situation in which the primary sporogenous cell functions as the megaspore mother cell, as is probable in most conifers.

GROWTH OF NUCELLUS.—By the formation of the integument the nucellus becomes limited to a knob, at first conical; but with the development of the megasporangium it soon becomes rounded. From the growth of the wall, as just described, there develops a considerable mass of tissue above the sporogenous tissue. At first this tissue seems to be uniformly meristematic, but later division becomes confined to the inner portions, the outer cells and the epidermis becoming radially elongated. I was not able to find any actual periclinal divisions of the epidermis, but the position of the cells in the layers next to the surface (fig. 65) would indicate such divisions as STRASBURGER (**36**) found in the development of the nucellus of *T. baccata*, giving a several-layered epidermis. The nucellus, therefore, is composed of two morphological entities,

the epidermis and the sporangium. The nucellus increases in diameter by anticlinal divisions of both epidermis and sporangium wall. Basal growth takes place also, so that the sporogenous region becomes situated in the focal center of the oval nucellus (figs. 40–42). From this time greater meristematic activity occurs in the peripheral regions contiguous to the line where nucellus and integument meet, resulting in the enlarged base of the nucellus. The tapetal function of that portion of the nucellus immediately surrounding the developing gametophyte, and the digestion of the nucellar tissue in the enlargement of the endosperm have already been described (12). The growing endosperm presses upon and stretches the nucellus so much that at maturity it is but a thin layer surrounding the endosperm.

A feature of interest is the extent of the freedom of the nucellus from the integument. In the earlier stages of development the two structures are entirely free from one another, a condition which persists until about the time of fertilization. The chalazal region now becomes the center of great meristematic activity, resulting in the development of the aril and the zonal growth of nucellus and integument as a united structure, so that at maturity the freedom of the nucellus from the integument is only partial. HOFMEISTER'S (14) statement that in *T. baccata* the separation between the "nucleus" (nucellus) and the integument extended entirely to the base was most probably based on young ovules. Freedom of nucellus and integument occurs in Paleozoic seeds belonging to the Cordaitales, such as *Cordianthus*, and is perhaps a primitive feature retained by most modern gymnosperms only during the early stages in the development of the ovule. That freedom of the two structures should persist longer in some forms than in others is not surprising, and has been regarded as having morphological significance. *Taxus*, *Torreya*, and some others are alike in retaining this feature for some time, the relative amount of it being correlated somewhat with the size of the seed, basal growth of the ovule being more extensive in some forms than in others. OLIVER (21) has called attention to the basal intercalary growth of the ovule in *Torreya*, which results in raising both nucellus and integument. He also suggests that the lower portion

of the seed is phylogenetically younger than the apex, where nucellus and integument are free from one another, introducing a problem already suggested by STRASBURGER (36) as to the real limits of the morphological ovule.

INTEGUMENT.—The development and structure of the integument of *T. baccata* have been described rather completely by STRASBURGER (35), BERTRAND (3), and JÄGER (15), and are not different in *T. canadensis*. The integument arises as a zone of meristematic tissue surrounding the young nucellus (figs. 37–39). Uniform growth in the entire zone results in a cylindrical, barrel-shaped integument surrounding the young nucellus (fig. 40), and extending some distance above it. At first the integument is uniform in thickness, six or more cell layers thick. The integument is 2-lipped from the early stages in its development, the lips alternating with the upper pair of scales. This feature has led some workers to interpret the integument as two carpels, and others as the fusion of two sporophylls. This 2-lipped character persists to the mature seed, but probably has no more morphological significance than has a similar and more pronounced feature in the ovules of many other conifers, especially the Abietineae, in which no foliar significance is attached to this character.

Up to the time of pollination the micropyle is relatively large (fig. 40). At pollination it is filled with the pollination droplet. At this time the inner wall of the integument is smooth, but soon after pollination becomes closed by the centripetal radial growth of a portion of the inner epidermis of two sides (figs. 41, 42, 66, 67). Closure of the micropyle in this way takes place even if the ovule is not pollinated, my preparations showing no difference in this respect between pollinated and unpollinated ovules. JÄGER found cases in *T. baccata* in which the micropyle had not yet closed at the time of fertilization, although usually taking place soon after pollination. In *Juniperus* both NORÉN (20) and NICHOLS (19) claim the failure of micropyle closing unless pollen of *Juniperus* has entered it, foreign pollen having no effect. Experimental data on this point would be of interest. It would seem that the pollination droplet would be a more likely growth stimulant in this region than the presence of a pollen grain on the somewhat distant nucellus,

or of pollen tubes within the nucellar tissue. JÄGER also speaks of a ring-formed thickening at the outer end of the micropyle, a feature not present in *T. canadensis*.

In its later development increase in thickness occurs below the tip region, while growth in length is largely the result of chalazal activity. In cross-section the young ovule is practically circular in outline, but as it develops it becomes more elliptical, and, especially in the upper portions, pronouncedly 2-ridged, the ridges corresponding with the lips. Frequently there are three ridges, occasionally four, the 2-lipped character, however, remaining constant. STRASBURGER records finding very rare cases of 5-ridged integuments. These ridges have been regarded as the midribs of fused sporophylls, but, as shown later, are associated with the vascular supply of the ovule and do not necessarily indicate a sporophyll character of the integument.

The histology of the integument has been accurately described for *T. baccata* by both STRASBURGER (35) and BERTRAND (3), a description which will also hold for *T. canadensis*. Before the hardening of the seed coat the following regions (fig. 68) are to be recognized: (1) the outer epidermis of large papillate cells, covered with a very heavy cuticle; (2) the hypoderm, large thick-walled cells, which become filled with brownish-red contents and give color to the seed coat; (3) a sub-hypodermal layer of small radially elongated cells; (4) a thick tissue of small irregular cells, extending to the inner epidermis, next to which the cells are longitudinally elongated; and (5) the inner epidermis, which in the micropyle region forms the plug tissue (fig. 67), and below, as far as free from the nucellus, consisting of elongated thick-walled cells containing a dark staining material. Below the union of the nucellus and integument the boundary between the two is not distinct. Large secretory cells are abundant in the inner tissue, and along the 2-keeled sides the strands of vascular elements traverse the integument. Formation of the stony character of the seed coat begins at the apex and extends downward, involving all the tissue of the integument excepting the epidermis and hypoderm, the cells becoming "stony," with very thick walls pierced by protoplasmic connections (fig. 69). The hardening begins very soon

after fertilization, and by seed maturity has reached the base of the seed. In the meantime the aril has developed, surrounding the hard nutlike seed.

Aril.—In the young ovule there is no indication of the aril, but about the time of pollination the aril primordium begins to develop as a ring at the base of the ovule (fig. 40). Its early development is contemporaneous with the chalazal growth of the ovule. In its early stages it is a flat saucer-shaped structure (figs. 5, 11) of greenish color and of slow growth until the seed is nearly matured and the seed coat hardened. Then there is very rapid growth; it soon becomes cup-shaped and reaches its mature condition, that of a large red fleshy cup inclosing the hard seed (figs. 8, 43). The chalazal portion is a tissue of small cells, traversed by the vascular elements which supply the hard integument. The sides of the aril consist of very large delicate-walled cells, filled with a watery material, the long cells being extended radially and obliquely upward. The epidermis is a narrow layer of small pigmented cells, and contains fairly numerous stomata, oriented longitudinally.

The morphological nature of the aril has been one of the mooted questions in the taxads, having been regarded as: (1) a special outgrowth surrounding the ovule, (2) a carpel, (3) representing the ovuliferous scale of other forms, (4) a second (outer) integument, and (5) the fleshy layer of a single integument. Richard (25) regarded the aril as the equivalent of the collar of *Ginkgo*, an accessory structure formed from the flower stalk. Blume (4) thought of it as a carpel, and Baillon (2) as an expansion of the axis surrounding the ovary. Parlatore (22) seems to have been the first to regard the aril as the morphological equivalent of the ovuliferous scale of other forms, a view followed by Celakovsky (8) and Worsdell (39), both claiming the ovuliferous scale of conifers to be the morphological equivalent of the "epimatium" of the podocarps, of the outer fleshy layer of the ovule of *Torreya* and *Cephalotaxus*, and of the aril of *Taxus*. Sinnott (33), in his study of the podocarps, holds a similar view with reference to *Cephalotaxus*, the logic of which would be to regard the aril of *Taxus* in the same light. Strasburger (35), with Baillon (2), regarded the aril

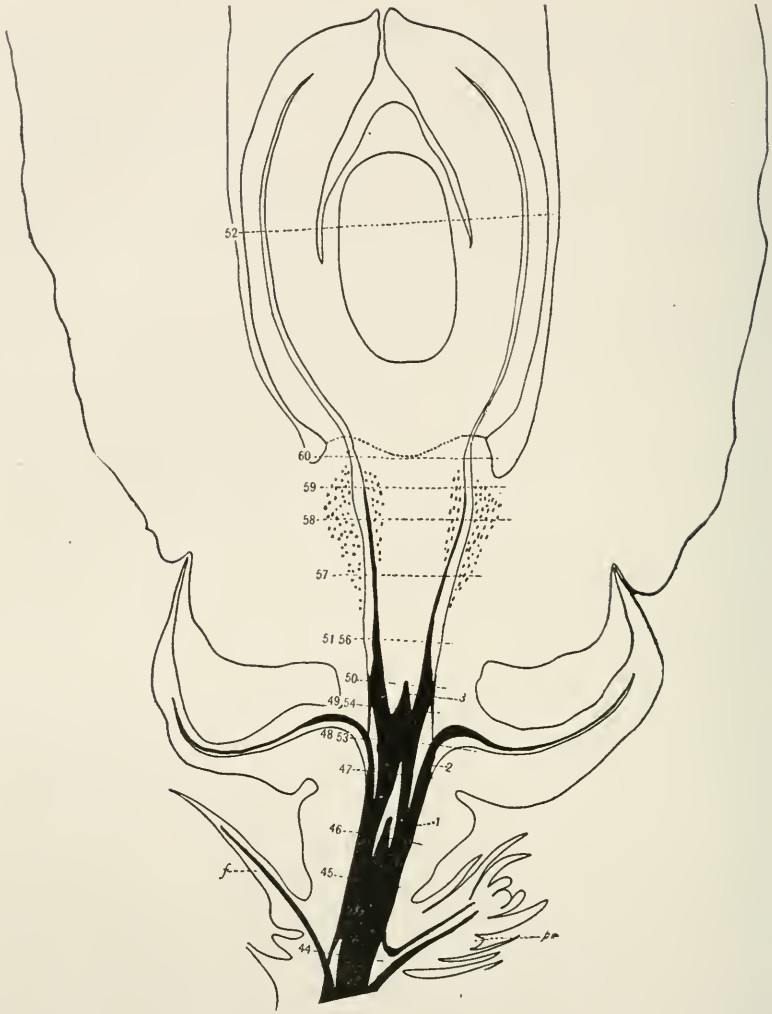


FIG. 43.—Semi-diagrammatic longitudinal section through primary shoot with secondary shoot and portion of mature ovule, ×17; outlines of primary and secondary shoots and aril of ovule made with camera lucida, ovule inserted diagrammatically; outlines of vascular supply also made with camera; note young ovule of next season above primary axis tip (*pr*); *1*, *2*, *3*, traces to 1st, 2d, and 3d pairs of scales, second pair of which shown in section; *f*, fertile scale of primary shoot; whole vascular cylinder of secondary shoot shown in black, light portions showing gaps in cylinder formed by scale bundles; in base of aril and seed, relation of xylem and phloem shown as seen in long section, xylem black, phloem white; scattered tracheids described are shown by area of black dots; curved line at base of seed shows line of separation when seed is removed from aril, and limit of camera outline of slide from which drawing was made; figures at ends of dotted lines across vascular tract indicate cross-section drawings corresponding to these levels.

as an outgrowth of the axis, discoid in nature, a view also held of the ovuliferous scale of other forms. BERTRAND (3) and SCHUMANN (31) both held the aril to be a special structure, the former regarding it as a proliferation of the cortical parenchyma at the base of the integument (which he regarded as the equivalent of the ovuliferous scale). JÄGER (15) regards the aril as a second or outer integument, basing his argument on the similarity in origin of the integument and the aril.

It will thus be seen that the structure is one which has given considerable difficulty in its interpretation, some of the explanations being perhaps more ingenious than reasonable. The carpellary nature of the aril no longer held sway after the acceptance of the gymnospermy of *Taxus*. That the aril may be a special structure arising from the axis and having no morphological significance seems an unnecessary way of avoiding the problem, and while possible is hardly probable. The view which regards it as equivalent to the ovuliferous scale of other forms has more in its favor, the chief objections to the idea for *Taxus* being the cauline origin of the ovule, independent of any recognizable sporophyll, and the belated appearance of the structure. It is hardly reasonable for the ovule to be present for so long and to reach such an advanced stage in development before the appearance of the structure on which it is supposed to be produced. Accepting the aril of *Taxus* and the fleshy layer of *Torreya* and *Cephalotaxus* as homologous structures, there is involved the difficulty of explaining why the aril should be free in one form and organically attached in the others, if representing the ovuliferous scale in all. The entire absence of a vascular supply in the aril of *Taxus*, excepting the strands which pass through its basal portion, makes impossible an interpretation based on its vascular features.

The question of two integuments or one seems to be partly a matter of terminology. Distinction needs to be made between the idea of two integuments, an inner and an outer one, and the idea of a single integument of three layers, the outer fleshy one of which may be more or less free from the other two. COULTER and LAND (10) have described the situation in *Torreya taxifolia*, and speak of the outer fleshy layer of the ovule as the outer integument. Concerning *Torreya*, COULTER and CHAMBERLAIN (9) state

that "it is a natural thing to see in these three layers characteristics of the testa in cycads, *Ginkgo*, and the older gymnosperms; and to conclude that the two integuments have arisen from a single one by delaying the development of the region that becomes the outer fleshy layer. These facts and the inference seem to hold good also in the case of *Taxus*, the only difference being that the outer fleshy layer (aril in this case) remains distinct from the inner one." In *Taxus* this freedom of the aril and hard integument extends to the base (fig. 43), probably due to the fact that the development of the aril begins relatively late. COULTER and LAND's figure of the ovule of *Torreya* at the mother cell stage shows considerable growth of the fleshy layer, while a corresponding stage (fig. 40) in *Taxus* shows but the beginning of the aril primordium. In *Torreya* there is a much greater and earlier chalazal growth of the ovule, resulting in a larger seed than in *Taxus*, the bulk of which is produced below the point of juncture of the fleshy layer and the hard coat.

In *Taxus* the inner fleshy layer may be represented only by the inner epidermis, and possibly a few layers of cells in the basal portion of the ovule, and is practically absent. The remainder of the seed coat becomes hardened, with the exception of the epidermis and hypoderm. It hardly seems reasonable to regard these two layers of cells as representing the outer fleshy layer, but rather that their failure to develop the stony character is due to their superficial position. "The probability is that the stony layer would not develop superficially in any event, so that it would not be necessary to regard a layer or two of cells overlying it (the hard coat) as representing the outer fleshy layer (COULTER and CHAMBERLAIN **9**, p. 418). The inference is that the outer fleshy layer is lacking in the Pinaceae, and from the same reasoning the outer layer of the seed coat in *Taxus* need not be regarded as an outer fleshy layer. Even the claim for two integuments in the old Cordaitean seeds is based on weak evidence, and the seed coat there "may correspond to the outer fleshy layer and stony layers of the single integument of cycads and *Ginkgo*" (COULTER and CHAMBERLAIN **9**, p. 174). SCOTT (**32**) also calls attention to the possibility of this view. It is likely that only a single integument

occurs in all known gymnosperms, excepting the Gnetales. In the older forms it is more or less distinctly differentiated into the three layers; in the modern forms one or more layers become "reduced," as the outer fleshy layers in most conifers and the inner fleshy layer in such forms as *Taxus*. On the other hand, the taxads are pronounced in the retention of the outer fleshy layer, *Cephalotaxus*, *Torreya*, and *Taxus* showing an excellent series both in the delay in appearance and in the freedom from the stony layer, *Taxus* showing both these features in greatest degree.

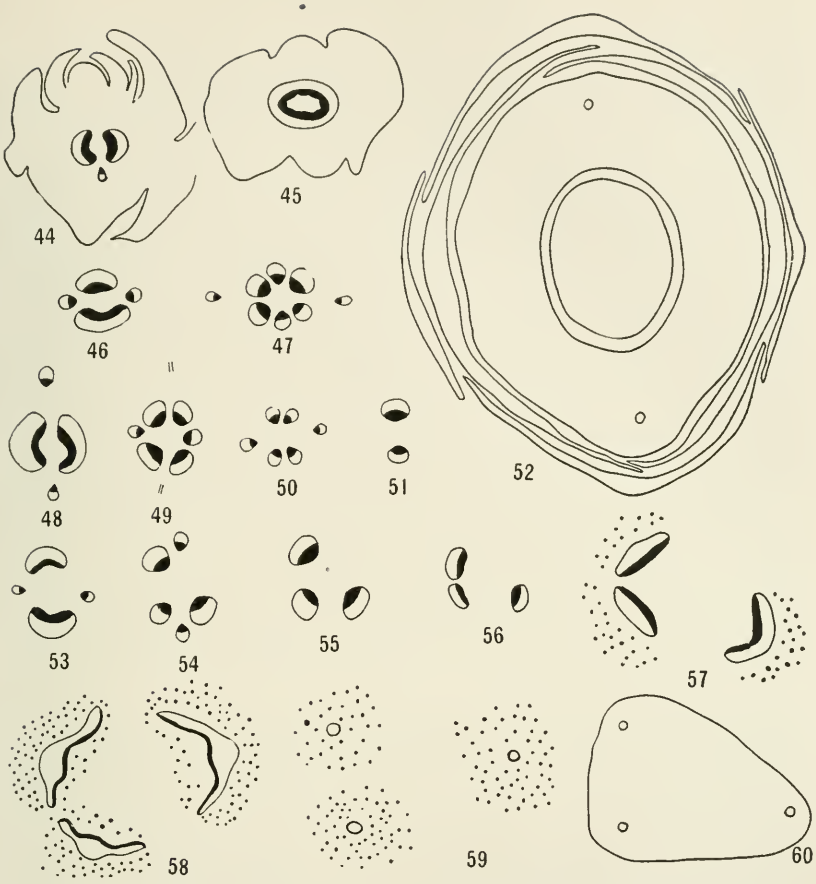
Attempts have been made to relate the taxads to the cycads on account of the fleshy character of the ovule, regarding *Cephalotaxus* and its relatives as bridging from cycads to conifers. The cycadean origin of the conifers does not harmonize with the known facts, however, and the attempt to relate all gymnosperms with fleshy seeds in a common phylogeny is almost as absurd as to attempt to construct a human "family tree" on the same basis. The tendency to "fleshiness" is too scattered to have any phylogenetic significance in a broad sense, although it probably has value within the narrower limits of small groups.

Vascular features

The vascular supply of the secondary shoot of *T. baccata* has been described by Van Tieghem (37), Strasburger (35, 36), and Miss Aase (1). Van Tieghem was the first to apply anatomical criteria to the morphological nature of the ovule, and concluded from the origin, orientation, and structure of the vascular supply that the ovule is a lateral structure, representing the first and only leaf of a branch of the third order arising in the axil of the "sixth scale" of the secondary shoot. According to his description, after the fertile scale has received its vascular supply, two bundles leave the axis, turn in such a way that the xylem is oriented outward, and these two bundles then penetrate the ovule, where, after forming a "small vascular cup," they give off, ordinarily two, sometimes three, or even four or five, branches into the integument. He also gave the bilateral symmetry of the ovule as one of the reasons for regarding it as axillary, bilateral symmetry being characteristic of leaf structures as contrasted with stem

structures. STRASBURGER (35) described the bundle supply to the three pairs of decussate scales and to the ovule, accepting VAN TIEGHEM's interpretation of the situation. Later he reversed his earlier view and regarded the ovule as terminal, there being nothing in the course of the bundles to give a clue to the lateral position of the ovule. He described the bundles in the integument as consisting of long, thin-walled elements, but containing no tracheids. Miss AASE describes the vascular supply to the ovule and the fusion in pairs of the four bundles from the axis as different from cases in which the united bundle is to supply an axillary structure, the pair consisting of "one bundle from each side of the bract bundle of the next lower pair, and not one from each side of the bract of the last pair." Miss AASE also pointed out the concentric character of the bundles in the base of the ovule, and the possible ending of one of the bundles before reaching the ovule. From her study the suggestion is made that there may have been a fusion of sporophylls to form a single structure, implying "the reduction of the ovules to one, the complete fusion of two sporophylls to the integument of the ovule, and finally the reduction of the vascular supply to each sporophyll to the single weak bundle in the wing of the ovule." She concludes, however, that "further investigation is necessary."

In *T. canadensis* the essential facts are not materially different from those of *T. baccata*, and a brief statement of the situation will be sufficient. The secondary axis receives two large bundles from the cylinder of the primary shoot (figs. 21, 44), these uniting at their edges and forming a closed cylinder (fig. 45). The traces to the first pair of scales are given off near this level (fig. 46). Traces are then given off to the second pair of scales (fig. 47), above which the gaps formed by the first pair of traces are closed, giving again two large bundles in the cylinder (fig. 48). The bundles to the third pair of scales are given off directly above those to the first pair (fig. 40), these bundles being usually quite short, at times not even reaching to the scale, but ending in the cortex itself. The main cylinder now consists of four bundles, two on each side, the pairs being separated by the gaps formed by the third pair of scale bundles. The two bundles of each pair turn through an angle of 45° and unite laterally (fig. 51), closing the gap formed by the second



FIGS. 44–60.—Figs. 44–52, series of transverse sections through young ovule (about age shown in fig. 11) showing normal vascular situation at various levels, corresponding to dotted lines figured in mature ovule of fig. 43; fig. 44, two bundles from primary shoot; fig. 45, closed cylinder; fig. 46, bundles to first pair of scales; fig. 47, bundles to second pair of scales; fig. 48, cylinder above second pair of scales; fig. 49, bundles to third pair of scales; fig. 50, cylinder of four bundles in base of ovule; fig. 51, two bundles resulting from pairing of four cylinder bundles; fig. 52, cross-section of young ovule, showing two vascular strands in integument and cyclic arrangement of three pairs of scales; ×24.

FIGS. 53–60.—Series of sections through mature secondary shoot and base of aril showing vascular supply to 3-ridged integument and relation of xylem and phloem in mature condition (note corresponding levels in fig. 43); fig. 53, bundles to second pair of scales; fig. 54, to third pair of scales, one of four bundles of normal cylinder lacking; figs. 55–57, each of three bundles remaining distinct, becoming broader tangentially at higher levels, and in fig. 57 showing scattered tracheids outside phloem; fig. 58, concentric bundle with narrow zone of continuous xylem next to phloem; fig. 59, concentric bundle consisting of small phloem strand surrounded by scattered tracheids; fig. 60, three phloem strands as they pass from aril to seed; ×24.

pair of scale bundles. At the base of the ovule there are then but two bundles, with xylem and phloem in normal position, and not showing the inverse orientation claimed for *T. baccata* by VAN TIEGHEM. Miss AASE's figures of *T. baccata* also show normal orientation at this level. These two bundles become more widely separated and enter the integument at opposite sides (figs. 43, 52), whence they traverse the integument almost to the tip of the ovule, their position being indicated externally by the ridges on the integument. As Miss AASE pointed out, one of the four bundles may terminate before reaching the base of the ovule (figs. 53–56), in which case the odd bundle may behave in the same way as the fused bundle. Ovules with three or four vascular bundles in the integument occur with some frequency, such situations occurring as a result of the failure of the fusion of one or both bundles, in which case each bundle is continued into the integument (figs. 53–60). Frequently when one of the four bundles of the normal cylinder is absent (figs. 54, 55) a 3-ridged integument results, no fusion taking place, but each bundle remaining distinct (figs. 53–60).

At the level of fusion the bundles are oval (fig. 51), and the fusion bundle remains this shape for some distance into the chalaza of the ovule. At a higher level they begin to widen laterally (figs. 57, 58), whether fusion has taken place or not, until near the upper level of the chalaza they reach their greatest width, both radially and tangentially. They then suddenly become narrow, and pass into the hard integument as narrow strands (figs. 43, 60). The bundles are endarch throughout their course, and at the base of the aril are collateral. Higher up, however, scattered xylem elements, consisting of short spiral-marked tracheids with bordered pits, appear outside the phloem (figs. 57, 58), and in the upper portions of the aril base the bundles consist of the phloem strand surrounded on all sides by the loosely distributed short tracheids (fig. 59). The tracheids occur only in the aril portion of the chalaza, the bundles as they pass into the integument consisting only of few thin-walled elements of phloem tissue.

It would seem that the vascular supply to the ovule favors the interpretation of it as terminal and cauline in nature. The vascular

supply arises equally from the two sides of the axis cylinder, the entire cylinder being involved in the supply. The bundles as they pair and fuse arise from opposite the second pair of scales and alternate the third pair of scales, an anomalous situation if the ovule were axillary to either of the third pair of scales. The ovule bundle supply is a direct continuation of the axis cylinder, the fusion of the bundles in the base of the aril closing the gap above the second pair of scale bundles. The orientation of the bundles is normal and presents no difficulty. The course of the bundles being opposed to the idea of an axillary origin is also against the view that there may have been a fusion of sporophyll with integument, and that the integumentary bundle is a vestige of that fusion. The presence of vascular bundles in the integument of gymnosperms is sufficiently common to cause no surprise in such forms as the taxads, nor is there any more argument for the sporophyll nature of the integument there than there might be in the cycads, where sporophyll and ovular integument are not confused, unless it be necessary to supply a theoretical sporophyll for a terminal cauline ovule.

The terminal cauline nature of the ovule is a much simpler interpretation of the facts, according both with the ontogenetic origin and the vascular supply. While this is an unusual situation for a gymnosperm, it is not out of harmony with a tendency among the seed plants, a tendency expressing itself frequently in angiosperms and not necessarily impossible in gymnosperms.

Summary

1. The ovuliferous bud arises in the axil of a leaf early in the season, and matures the next year.

2. The ovuliferous organ consists of the primary shoot and the secondary shoot with the ovule.

3. The primary shoot is to be regarded as a vegetative branch of limited growth, bearing only reproductive axes (secondary shoots). While of limited character, at times it may become a functional vegetative shoot like any other vegetative branch.

4. The primary shoot is a persistent structure, functional for several successive seasons.

5. Occasionally the primary shoot may be terminal to a leafy branch.

6. The secondary shoot consists of three pairs of decussate scales and a terminal ovule.

7. The ovule arises as a direct continuation of the axis, there being nothing in its origin to indicate that it is a lateral structure.

8. The archesporium arises from the hypoderm. The sporogenous tissue consists of a considerable mass of cells, out of which one or two may function as megaspore mother cells.

9. The aril is regarded as the morphological fleshy layer of a 3-layered seed coat, delayed in appearance and physically separate from the hard stony layer.

10. The ovule receives its vascular supply direct from the axis cylinder, contrary to any axillary nature, and in harmony with the view that it is a cauline structure.

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EXPLANATION OF PLATE XXIII

All figures were made with a camera lucida excepting figs. 2, 4, 8, 10, and part of 43. Text figures have been reduced to one-third and plate figures to one-half original size. The scale of magnification of the figures is shown in connection with the descriptions.

FIG. 61.—Archesporial initial showing hypodermal position; ×475.

FIG. 62.—Two archesporial cells divided, each forming primary wall cell and primary sporogenous cell; ×475.

FIG. 63.—Primary wall cells divided and beginning formation of megasporangium wall; ×475.

FIG. 64.—Older nucellus showing several-layered wall and central mass of sporogenous tissue (detail of fig. 39); ×475.

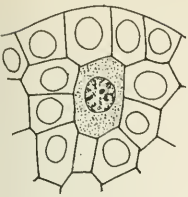
FIG. 65.—Portion of nucellus showing several-layered epidermis (cells without nuclei), megasporangium wall (cells with nuclei), and sporogenous tissue (shaded) with group of megaspores; ×475.

FIG. 66.—Portion of integument showing beginning formation of plug tissue; ×210.

FIG. 67.—Mature plug tissue; ×210.

FIG. 68.—Detail showing integumentary regions, outer papillate epidermis with heavy cuticle, hypoderm of large cells, sub-hypodermal layer, and internal tissue; inner epidermis not shown; ×210.

FIG. 69.—"Stony cells" from hard integument showing protoplasmic connections; ×210.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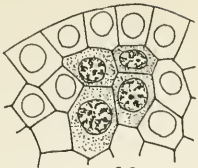
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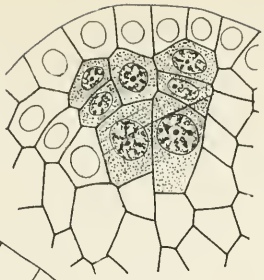
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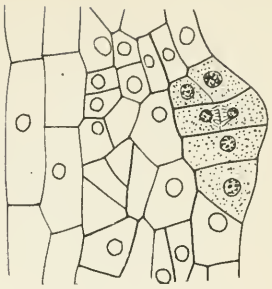
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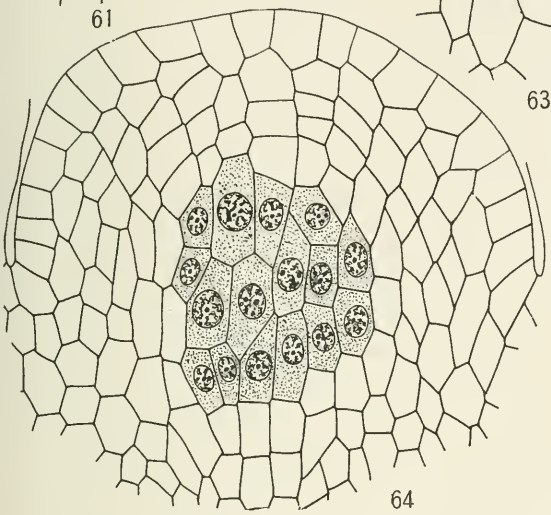
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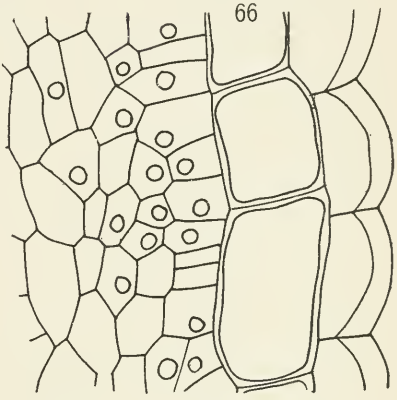
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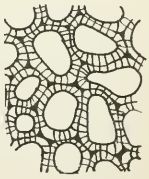
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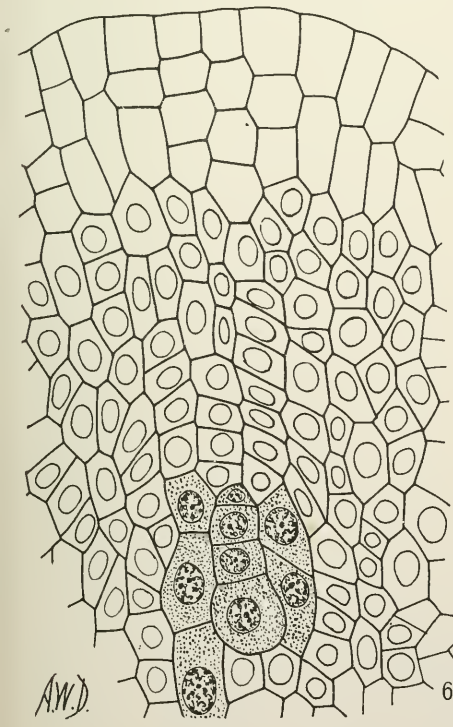
65

67

GEO-PRESENTATION AND GEO-REACTION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 268

(WITH FIVE FIGURES)

EVA O. SCHLEY

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THE BOTANICAL GAZETTE, Vol. LXX, No. 1, July 1920

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EVA O. SCHLEY

(WITH FIVE FIGURES)

Historical

The reaction of plants to geotropic stimulation has been the subject of considerable investigation, the problem having been attacked from many standpoints. Naturally, perhaps, the physical side was studied first, a number of workers having developed the main features of gravity stimulus, presentation and reaction times, perception, conduction and response, organs of perception, and other related subjects. The chemical side of the field, involving the change in metabolism of the stimulated organ, has received much less attention.

The first worker in this field seems to have been KRAUS. As early as 1870 he published (14) the first of a series of researches on the chemical content of the growing plant, both in normal relations and after subjection to various external stimuli. This research included (1) the water content, (2) the acidity, (3) the sugar content of the normally growing shoot, (4) the relation of each to the growth maximum, and (5) steps in the change of the cell content of the concave and convex side of the geotropically and heliotropically responding organ. He determined that, in the normally growing shoot, (1) the acidity decreases from the tip downward, (2) the water increases relatively from the tip to the downward limit of growth, and (3) the sugar increases from the tip below the growth maximum and therefore is not a limiting factor in growth. In the stimulated organ he found on the convex-becoming side (1) an increase of sugar production up to the time of visible curvature and then a decrease, (2) a progressive decrease in acidity during stimulation, free acid being entirely absent from the responded organ, and (3) a progressive increase of water preceding curvature.

DeVries (5) studied the forces released in gravity stimulus, the effect of these released forces upon curvature, and the release of elasticity by gravity stimulus. He concludes that gravity produces an increase of osmotically active material in the cells of the convex-becoming side, causing an intake of water from the adjacent tissue, the resulting increased turgor producing a longitudinal extension of the elastic cell membranes, which, originally plastic, become fixed through growth and lignification.

Cielsielski (2) observed a difference of the cell sap on opposite sides of geotropically stimulated roots, the cells of the convex-becoming flank exhibiting a thin watery protoplasm in contrast with the denser, more opaque plasma of the side becoming concave. Kohl (13) obtained analogous results in the sporangiophores of *Phycomyces*, in that in geotropic stimulation the plasma of the concave side of the filament became much thicker, while that of the convex side became thin and watery. He concluded that there was a causal relation between this differentiation of cell plasma and the curvature of the organ. Elfving (7), however, according to his reviewers, produced a similar differentiation of protoplasm in *Phycomyces* sporangiophores by allowing them to push against a glass obstruction, a purely mechanical stimulation.

Hilberg (11), contrary to DeVries' results, found that in geotropic stimulation the osmotic pressure of the concave side of leaf joints and stem nodes of various plants is greater than that of the convex side. Wortman (22) negatives both DeVries' and Hilberg's conclusions, since he could find no difference in the osmotic pressure of the two flanks of stimulated organs, and holds DeVries' view of the causal relation between turgor and curvature to be wholly untenable. On the other hand, he agrees with Kohl in that he found in geotropically stimulated organs the plasma "wandered" from the convex to the concave side, the thickened plasma inducing the cell membranes of the concave side to become thicker but less elastic and less extensible than those of the convex side. These latter, stretching longitudinally, force the concave side upward, thus producing curvature. Noll (16) confirms Wortman's work, but refutes his argument of the causal relation between the changed activity of the plasma and the curvature of the organ.

COPELAND (3) was unable to detect a difference in the opposite flanks of stems split lengthwise and stimulated geotropically four days. KERSTAN (12), using the plasmolysis method, found no increase of turgor in either flank of geotropically or heliotropically stimulated shoots either during curvature or after its completion. He concludes with NOLL that the decrease of turgor is due to the fact that the osmotic producing substances do not keep pace with the intake of water of the cells and their increased volume.

THATE (20) found KRAUS's method too crude to determine the difference of water in the two flanks of heliotropically stimulated shoots, although he does not dispute its existence. On the other hand, TONDERA (21) was able to verify KRAUS on this point and from his studies developed the law: "As the cells of the rind parenchyma of the lower organ half become filled by the streaming of water, due to gravity, the cells of the opposite half become water-poor, the resulting difference in pressure forcing the organ to move toward the water-poor half." This at best is a very crude conception.

From the cytological standpoint, MCDOUGAL (15) found that the cells of the convex side are greater in length, breadth, and thickness than those of the corresponding tissue of the concave side of geotropically stimulated roots. GEORGEVITCH (8) confirms this earlier work, while BÜCHNER (1) found the same condition in shoots that had been prevented from responding to gravity stimulation.

CZAPEK (4) is probably the chief worker in the chemical field of geo-presentation and reaction. Working with normal seedlings, he found that homogentisic acid is produced as a product of the oxidation of tyrosin, through the action of an oxidase, tyrosinase. In geotropic stimulation the tyrosin is converted into homogentisic acid by tyrosinase, as in normal seedlings, but the further oxidation of the homogentisic acid by the oxidase is inhibited by the production of an anti-oxidase, which renders the oxidase partly ineffective and by this means causes an accumulation of homogentisic acid. The accumulation begins after five minutes' stimulation, reaches a maximum at the time of distinct curvature, and disappears when reaction is complete. GROTTIAN (10) and GRAFE and LINSBAUER (9), however, were unable to confirm his results. They found as

great variation in the amount of homogentisic acid in different analyses of normal shoots as CZAPEK found between the stimulated and unstimulated organs.

In her work on thermotropism of roots, ECKERSON (6) found the greater permeability to be on the concave side of the root, and that this permeability changed with the changed thermotropic reaction of the root.

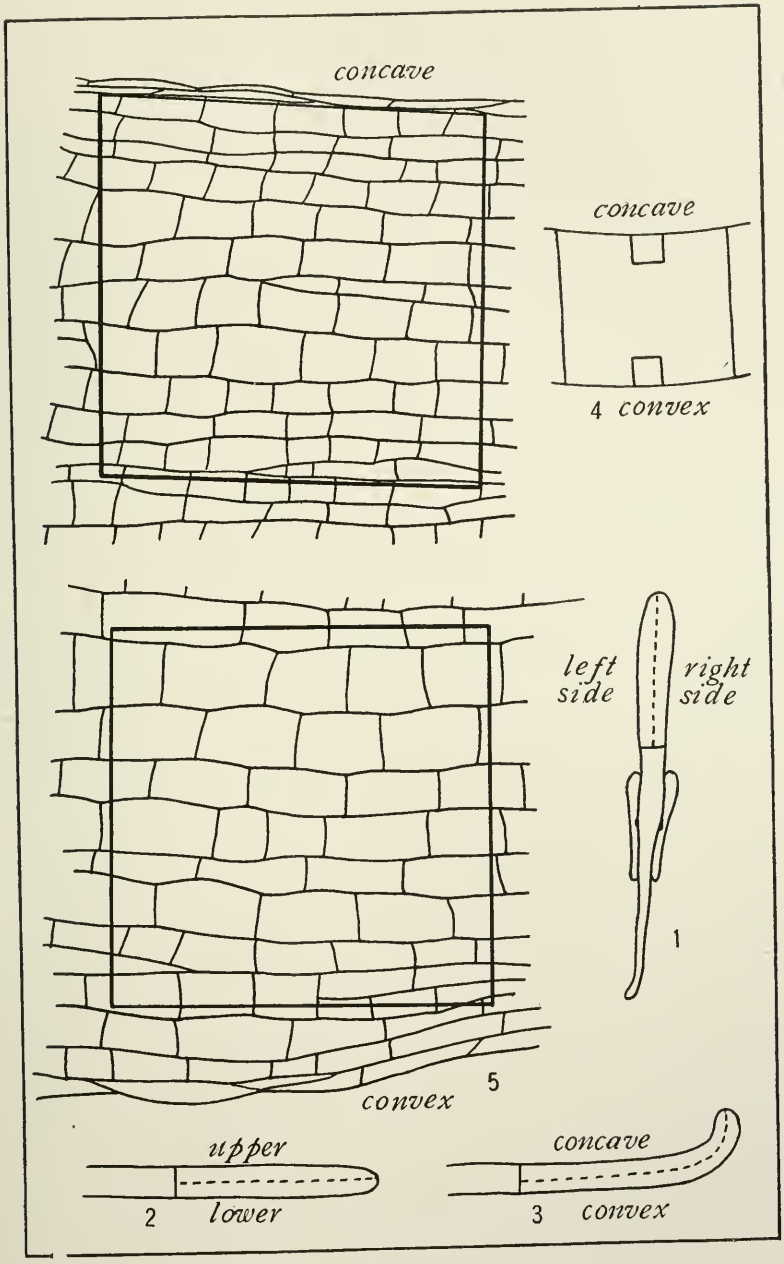
Scope of experiment

The work here presented is a continuation of that reported in a previous paper (18), which dealt with the acidity of the normal shoot, and compared the acidity of the two flanks of the geotropically stimulated shoot. The present paper deals with the changes in metabolism of the carbohydrates, the difference in osmotic pressure, and the difference in respiration of the upper and lower flanks of the geotropically stimulated shoot through presentation and reaction periods. *Vicia Faba* seedlings were employed throughout the experiment because they respond readily to geotropic stimulation, and because they are large enough to be easily split longitudinally. Trouble was experienced in germinating the seeds because of the development of mold. To overcome the difficulty the seeds were washed in tap water and then soaked two or three minutes in a 1 per cent solution of silver nitrate and rinsed thoroughly. They were grown in sand which had been sterilized by boiling in water an hour or longer, and then put into sterile pots while hot and allowed to stand until the following day before planting the seeds.

Carbohydrates and proteins

For this analysis etiolated seedlings of *Vicia Faba*, grown in sand in the greenhouse at a temperature of about 20° C., were used. When the seedlings were 6–8 cm. high they were geotropically stimulated for periods ranging from 15 minutes to 5 hours. Duplicate analyses of samples for each period of stimulation, as well as duplicate analyses of the unstimulated organ as controls, were made.

The epicotyls were split longitudinally into right and left halves (fig. 1) in the controls, and into upper and lower halves


concave
concave
4 convex
left side
right side
1
convex
5
upper
2 lower
concave
3 convex

FIGS. 1–5

(fig. 2) in the stimulated seedlings. These latter become the concave and convex halves (fig. 3) respectively in the responded organ. Samples varying from 6 to 10 gm. were used. The fresh portions were weighed in weighing bottles and the weight obtained by difference. The tissue, thus obtained, was cut up fine and triturated, killed in boiling 85 per cent alcohol, and boiled for 30 minutes.

The triturated tissue was subjected to alcoholic extraction for 3 hours and to ether extraction for 2 hours in the Koch modification of the Soxlet extractor. The tissue was then pulverized and extracted in boiling water 30 minutes. This water extraction was repeated six times. Following this was another alcohol extraction of 24 hours. The original killing alcohol, the ether extract (the ether was evaporated and the extract brought into solution in water), the water extract, and the two alcohol extracts were combined, and the volume increased to 500 cc. by the addition of water. This extract contained all the material soluble in these solvents, that is, the sugars, the lipoids, and the amino acids, and may be designated F_1. The residue contained the insoluble substances (starches, pectins, hemicelluloses, and cellulose), and may be designated F_2. From F_1 was taken three 150 cc. portions for the determination of (1) sugar, (2) nitrogen, and (3) dry weight. From F_2 was obtained (1) the dry weight and (2) the hydrolyzable polysaccharides.

The alcohol-water-soluble portion for the determination of sugar was freed from alcohol by evaporation on the steam bath, water being added before and during the process of evaporation, and the final volume brought to 150 cc. The tannins and lipoids were precipitated by the addition of a 10 per cent solution of basic lead acetate, the volume made up to 200 cc. and filtered immediately. The excess lead was precipitated from 150 cc. of the filtered solution by means of a saturated solution of ammonium sulphate, and the volume brought again to 200 cc. Duplicate determinations of 50 cc. portions were made of sugar solutions thus obtained, by the Munson and Walker method for the determination of reducing sugars (17). The amount of cuprous oxide thus obtained was determined by the volumetric potassium permanganate

method (*op. cit.*, pp. 52, 53.). An N/20 solution of permanganate was used. A third 50 cc. portion of this clarified solution was used for the determination of non-reducing or hydrolyzable sugars. The hydrolysis was performed according to the method for the determination of sucrose in the absence of raffinose (*op. cit.*, pp. 40, 41). The cooled solution was neutralized with 20 per cent sodium hydrate, brought to 100 cc. volume, and duplicate sugar estimations made of 50 cc. portions as described.

The residue (F_2), after the determination of the dry weight (to be described later), was used for the determination of the polysaccharides according to the method for direct acid hydrolysis of starch (*op. cit.*, p. 53). Duplicate determinations of 50 cc. portions were used for the determination of sugars, as in F_1.

The calculations were based on the milligrams of copper oxidized in the change from cuprous to cupric oxide, and expressed in equivalent milligrams of dextrose obtained from the Munson and Walker table accompanying the method of analysis (p. 243).

The portion of F_1 for the determination of dry weight was evaporated to moist dryness on the steam bath and brought to constant weight in vacuo. The dry weight of F_2 was obtained by bringing the residue of the original tissue to constant weight in the electric oven at a temperature of 104° C. The calculations for both were based on the dry weight per gram of the fresh material.

The third portion of F_1 was used for the determination of the total nitrogen. This determination was made after the Kjeldahl method as modified by Arnold. Calculations were made on the amount of nitrogen per gram of fresh weight. Table I shows the results obtained. It will be noticed that the soluble sugars vary but little throughout. The hydrolyzable sugars increase markedly at the time of visible response, and are greater on the convex side. The polysaccharides decrease as the hydrolyzable sugars increase. The dry weight of F_1 remains practically constant. The dry weight of F_2 remains practically constant until the beginning of curvature, when the weight of the convex side becomes less. The results of this sugar determination are not comparable with the work of Kraus, for he was working with the raw pressed sap, which probably contained reducing substances other than sugars, as he himself suggests.

KRAUS found that the reducing substances increased on the convex side of the responding organ up to the time of visible curvature, and then decreased on that side of the curved shoot, a point upon

TABLE I

DETERMINATION OF MATERIAL PER GRAM FRESH WEIGHT

TIME STIMULATED	F_1 Soluble sugar (mg.)	F_1 Hydrolyzable sugar (mg.)	F_2 Polysaccharide (mg.)	F_1 Total carbohydrates (mg.)	F_1 Dry weight (g.)	F_2 Dry weight (g.)	F_1 Total nitrogen (g.)
Sample I, unstimulated							
8.74 (right)	23.45	3.21	3.60	30.26	0.06845	0.01920	0.004800
9.35 (left)	23.30	3.95	3.31	30.56	0.06878	0.01876	0.004535
Sample II, stimulated 15 min.							
11.88 (upper)	23.58	2.96	5.75	32.29	0.07124	0.02449	Lost
12.05 (lower)	22.20	3.10	4.93	30.23	0.06894	0.02176	Lost
Sample III, stimulated 30 min.							
10.38 (upper)	19.45	3.54	3.37	26.36	0.06460	0.01809	Lost
10.38 (lower)	19.35	3.58	3.02	25.95	0.06888	0.01887	Lost
Sample IV, stimulated 1 hour							
11.58 (upper)	22.44	4.94	4.00	31.38	0.06296	0.01994	0.004346
12.20 (lower)	22.00	4.73	2.98	29.71	0.06621	0.02014	0.004353
Sample V, stimulated 1.5 hours							
7.17 (upper)	21.90	6.34	4.72	32.96	0.05788	0.02224	0.004238
7.09 (lower)	21.62	4.82	4.66	31.10	0.06379	0.02146	0.004448
	Beginning visible response						
Sample VI, stimulated 2 hours							
10.23 (upper)	18.65	6.20	2.44	27.29	0.05803	0.01828	0.003697
10.15 (lower)	21.50	9.15	3.66	34.31	0.06318	0.01832	0.003838
Sample VII, stimulated 3 hours							
9.32 (upper)	23.10	8.34	3.29	34.73	0.06716	0.02054	0.004421
9.05 (lower)	28.25	9.10	3.50	40.85	0.06802	0.01950	0.004738
Sample VIII, stimulated 4 hours							
9.69 (upper)	23.05	4.90	3.44	31.39	0.07426	0.02100	0.003985
9.66 (lower)	23.45	7.86	3.18	34.49	Lost	0.01875	0.004144
Sample IX, stimulated 5 hours							
6.92 (upper)	21.10	9.20	1.88	32.18	0.06713	0.01907	0.004855
7.63 (lower)	26.75	13.82	2.62	43.19	0.06783	0.01686	0.004593

which he has frequently been misquoted (**14**, pp. 87 and 89). The total nitrogen remains constant throughout, the unstimulated sample I showing almost identically the value of sample IX at the close of the experiment.

Osmotic pressure

The osmotic pressure of the two flanks during the period of presentation and response was determined by means of plasmolysis. Weight molecular solutions of cane sugar and potassium nitrate were used as plasmolyzing agents. The seedlings were geotropically stimulated for varying periods of time. Portions of the seedlings, including the region of response, were sectioned (on the hand microtome) vertically, that is, from upper to lower side of the horizontally placed shoot. The sections were placed in weight molecular solutions of the plasmolyzing agent of such percentages as previous experiment had shown to be close to the plasmolyzing point. The series of weight molecular solutions was graduated to intervals of one-half of 1 per cent. The accompanying tables and graphs show the results of one each of the experiments made.

PLASMOLYSIS

(Using cane sugar as plasmolyzing agent)

In normal shoots both sides plasmolyze at 42 per cent weight molecular
After 5 minutes' stimulation both sides plasmolyze at 42 per cent
After 10 minutes' stimulation both sides plasmolyze at 43 per cent
After 45 minutes' stimulation upper side at 43, lower side at 44 per cent
After 1.5 hours' stimulation upper side at 43, lower side at 44.5 per cent
After 5 hours' stimulation both sides at 43 per cent

(Using potassium nitrate as plasmolyzing agent)

In normal shoots both sides plasmolyze at 31 per cent weight molecular
Stimulated 15 minutes both sides plasmolyze at 31 per cent
Stimulated 30 minutes both sides plasmolyze at 32 per cent, upper general, lower a few cells
Stimulated 45 minutes upper faintly at 33, lower at 33.5 per cent
Stimulated 1.25 hours upper at 32, lower at 33 per cent
Stimulated 5 hours both sides at 32 per cent

These results indicate that the osmotic pressure of the cell rises as the time of stimulation increases, reaches a maximum at or before visible response, and decreases as the response nears completion. The osmotic pressure is greater on the convex side during the period of response. It is interesting to note that the maximum acidity, as shown in a previous paper (18), is reached in 30 minutes, while the maximum turgor is reached in 45 minutes. The results of different investigators on the turgor change of stimulated shoots

show little agreement. Some writers have found the greater turgor on the concave, some on the convex, side; others have found no difference in turgor in either flank of the stimulated shoot or in the stimulated versus the unstimulated organ, with the balance of the argument rather in favor of the last mentioned. Inspection of the work of those writers who have tabulated their results, however, shows that the time of stimulation (ranging in general from several hours to several days) was too long to catch the change in turgor, which change appears to take place in a relatively short time, as was originally determined by DEVRIES in his macroscopic turgor experiments on geotropically stimulated grass nodes.

Respiration

Qualitative experiments were conducted upon the relative respiration of stimulated and unstimulated roots, and upon the upper and under flanks of geotropically stimulated shoots. These experiments were made in the TASHIRO (19) biometer apparatus, which determines the relative rate of respiration by the precipitation of barium carbonate on the surface of a drop of barium hydrate in a closed chamber.

The roots, without previous stimulation, were placed, one horizontally and one vertically, in similar chambers designated as left and right respectively. The shoots were stimulated for periods varying from 10 minutes to 5 hours. They were split longitudinally just before being placed in the apparatus. The roots were suspended and the shoots were placed horizontally, the upper with the cut surface down, and the lower with the cut surface up, as during stimulation. Both were placed across Van Tieghem cells in order to give equal opportunity for carbon dioxide diffusion. Many seedlings were tested with uniform results.

Table II shows that a geotropically stimulated root has a higher rate of respiration than the unstimulated root, and that in the stimulated shoot the under (convex) side shows a higher rate of respiration than the upper (concave) side at all intervals of time during stimulation and response that were investigated. It also shows that the rate of respiration decreases as the time of stimulation increases.

The effect of geotropic stimulation upon the cell structure of the responded shoot was determined through microscopical examination. Longitudinal sections from concave to convex side of the completely responded shoot in the region of the angle of greatest curvature were cut on the freezing microtome, and camera lucida drawings were made of corresponding areas on the concave and convex flanks of the organ (figs. 4, 5).

TABLE II

RELATIVE RESPIRATION

Seedling	Left chamber	Right chamber	Time stimulated	Time in apparatus	Greater precipitation of $BaCO_3$
Roots					
Sunflower...	Horizontally placed	Vertically placed	.....	4 minutes	Horizontal root
Sunflower...	Vertically placed	Horizontally placed	.....	4 minutes	Horizontal root
Zea Mays...	Horizontally placed	Vertically placed	.....	4 minutes	Horizontal root
Shoots					
Vicia Faba..	Convex side	Concave side	10 minutes	2 minutes	Convex (much greater)
Vicia Faba..	Convex side	Concave side	2 hours, 31 minutes	3 minutes	Convex side
Vicia Faba..	Concave side	Convex side	4 hours, 58 minutes	7 minutes	Convex side

A study of fig. 5 shows that the cells on the convex side are larger than those on the concave side. A 10 cm. square on the convex side contains 40 cells, while a corresponding area on the concave side shows 72 cells. This result is in accord with the work of previous investigators.

Summary

1. The reducing sugars remain constant throughout stimulation and response.

2. The hydrolyzable sugars increase on the convex side at the expense of the polysaccharides as response takes place.

3. The total sugars are constant until beginning of response, when the sugars of the convex side become greater.

4. The osmotic pressure increases until visible curvature has taken place. At the end of the reaction both flanks show the same osmotic pressure, which, however, is greater than that of the normal shoot.

5. Respiration of the geotropically stimulated root is greater than that of the unstimulated organ.

6. The rate of respiration of the convex side of the geotropically stimulated shoot is greater than that of the concave side throughout the period of perception and response.

7. Respiration decreases as the time of stimulation increases.

8. The steps, in point of time, of the chemical changes that take place in a geotropically stimulated shoot are: (1) increased respiration, (2) increased acidity (18), (3) increased turgor, and (4) increased production of hydrolyzable sugars with corresponding decrease of polysaccharides on the convex side of the responding organ.

The writer is greatly indebted to Dr. WILLIAM CROCKER, who suggested the problem, and who gave much assistance during the progress of the work; to Dr. F. C. KOCH for help in the methods of analysis; and to Dr. SHIRO TASHIRO for assistance in the work on respiration.

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STUDIES IN THE GENUS BIDENS. V

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 269

EARL E. SHERFF

(WITH PLATES XI–XIV)

Bidens exigua, sp. nov. (pl. XI).—Herba annua, 1.5–3 dm. alta; caule tenuissimo, subrecto, striato, subsimplici. Folia opposita (summa alternata), membranacea, petiolata, petiolo adjecto 3–5 cm. longa, bipinnata, glabra; foliolis (3 aut 5) maximam partem 3–5-partitis; lobis integris, subobtusis, infirme apiculatis. Petioli 0.5–2 cm. longi, basi connati. Capitula pauca aut solitaria, discoidea, tenuiter pedunculata pedunculis 2–5 cm. longis, ad anthesin 5–6 mm. longa et 1.5–2 mm. lata infra, 2–3 mm. lata supra; in fructu, circ. 9 mm. longa et 2–5 mm. lata. Involucrum basi sparsim hispidum aut glabratum; bracteis duplici serie dispositis; exterioribus (4–7) linearibus, 2–3 mm. longis, ciliatis, ad faciem glabris aut pubescentibus, ad apicem induratis; interioribus dimidio longioribus, glabratis, lanceolatis, striatis, margine diaphanis. Achaenia (submatura) linearia, glabra aut supra ad margines remote hispida, bi- aut triaristata aristis retrorsum hamosis hamis tenuibus, 4–8 mm. longa.

SPECIMENS EXAMINED.—*C. H. T. Townsend* 1513, alt. 1607 m., Chosica Canyon, Peru, April 20, 1913 (Herb. U.S. Nat. Mus., no. 602943, type).

The plants of the type sheet would seem at first to be merely depauperate or impoverished forms of some species normally larger and perhaps already described. The technical characters, however, do not match those of any other species known to me. There are several species very close to *B. exigua* all of

which might once easily have passed for *B. bipinnata* L., yet which, with subsequent advances in our knowledge, have been proved indisputably to be distinct and severally valid. Among these are *B. heterosperma* Gray and *B. Lemmoni* Gray of the southwestern United States and Mexico, and *B. parviflora* Willd. of Asia. *B. exigua* is nearest to *B. Lemmoni* and *B. parviflora;* like these, it may well be expected to prove constant and worthy of specific rank.

Bidens duranginensis, sp. nov. (pl. XI).—Herba annua, glabrata, demum circa 6–9 dm. alta; caule subtetragono, ramis acute tetragonis, ramis acute tetragonis, longis et tenuibus, striatis, infra minute pubescentibus. Folia opposita, petiolata, petiolo adjecto 10–12 cm. longa (eis ramorum 1–3 cm. longis), pinnata, serrata aut dentata (aut etiam inciso-dentata), ciliata, foliolis ovatis aut ovato-lanceolatis, saepe duobus aut quatuor imis cuiusque folii tripartitis. Petioli (foliorum caulis) 2.5–4 cm. longi, ad basim ciliati et connati. Capitula multa, subligulata, tenuiter pedunculata, pedunculis 3–8 cm. longis, ad anthesin 4–7 mm. alta et (ligulis adjectis) 0.8–1.3 cm. lata, in fructu 1.2–1.4 cm. alta et 6–8 mm. lata. Involucrum basi hispidum, bracteis duplici serie dispositis; exterioribus (circ. 8) linearibus, fere glabris, apice induratis, 2–3 mm. longis; interioribus dimidio longioribus, anguste lanceolatis; membranaceis, margine diaphanis. Ligulae (3–6) subalbidae, anguste ovatae, 4–7-striatae, 4–6 mm. longae. Achaenia linearia, nigra, glabra aut supra sparsim hispida, 2–4-aristata aristis flavis et retrorsum hamosis, corpore 6–12 mm longa.

Specimens examined.—*Dr. Edward Palmer* 756, west side of Iron Mountain, vicinity of city of Durango, Durango, Mexico, October 1896 (Herb. Gray, type; Herb. Field Mus., no. 51825; Herb. U.S. Nat. Mus.); *idem* 612, vicinity of city of Durango, Durango, Mexico, April to November 1896 (Herb. Field Mus., nos. 51704 and 51705; Herb. Gray, differing from type material, apparently, merely in being somewhat younger).

This species, if we attempt to delimit it in a taxonomic way, is confessedly of unsatisfactory status. The type collection had been determined as *Bidens anthriscoides* DC., but the plants are very different from the type material of that species (*Berlandier* 1010, Herb. Brit. Mus.; Herb. Drake, Paris). It is manifestly an ally of *B. pilosa* L., from which it differs in its whitish rays and in its foliage, the lower leaflets of the stem leaves tending to be distinctly tripartite. This last character distinguishes it likewise from *B. leucantha* (L.) Willd. In foliage characters it slightly simulates *B. chinensis* (L.) Willd. of

the Orient and *B. subalternans* DC. of South America. I have seen two specimens by E. O. Wooton from New Mexico (Mesilla Valley, Dona Ana County, October 1895, U.S. Nat. Herb., nos. 561445 and 663170; referred to *B. anthriscoides* DC. by Wooton and Standley, Contrib. U.S. Nat. Herb. 19:704. 1915) that are evidently true *B. bipinnata* L., yet which approach *B. subalternans* DC. A third plant, also by Wooton (Las Cruces, New Mexico, October 1895, Herb. N.Y. Bot. Gard.), approaches *B. subalternans* DC. in foliage still more, but is nevertheless clearly a form of *B. bipinnata* L. All three of these plants are suggested by the type of *B. duranginensis*. They appear, however, to be entirely distinct in a specific way. Future field studies, to determine the range of variation and the limits of demarcation for the Durango plants, are highly desirable.

No other group in the genus *Bidens* has been so badly neglected heretofore, considering the number of species involved, as has that group native to the Hawaiian Islands and other islands of the Pacific, and, by some authors, segregated as a separate genus, *Campylotheca*. Nearly a century ago Gaudichaud (Voy. Freycinet Bot. 464. *pl. 85*. 1826–1829), describing a species collected in the Hawaiian Islands during Freycinet's voyage, named the plant *Bidens micrantha*. Shortly afterward Cassini (Dict. Sci. Nat. 51:475. 1827) called attention to the curved achenes of Gaudichaud's species. He made this achenial character the basis for proposing his new genus *Campylotheca*. Later Lessing (Linnaea 6:508. 1831) accepted Cassini's genus for species like *Bidens micrantha* Gaud., but he erected a new genus, *Adenolepis*, to include a somewhat different form. I propose to discuss *Adenolepis* in a future article. Concerning *Campylotheca*, however, we may proceed to note that the name was retained by De Candolle in his *Prodromus* (5:593. 1836), although elsewhere it was accorded only slight attention. In fact, the collections in those days embraced so few specimens from the Pacific Islands that little study was made of the Pacific flora by taxonomists. Nuttall, in 1841 (Trans. Amer. Phil. Soc. N.S. 7:368), reduced *Campylotheca* to the rank of a section under *Bidens*, but did not give extended reasons for so doing. His attention had been directed to the subject by his having traveled among the Hawaiian Islands and discovered there at least one new species of *Bidens* (*B. gracilis*). Nuttall, however, did evince a rejection of Cassini's main character for *Campylotheca*,

namely, the curved or twisted achenes. He worded his description to read "sometimes curved or contorted," and for one species (*B. mutica*) he definitely described the achenes as "straight." Since NUTTALL'S time, we may add, many other closely affiliated species have been discovered, including forms of *Bidens micrantha* itself, which have straight achenes, thus bringing the curved-achene character into discredit. In 1856 SCHULTZ BIPONTINUS undertook the determination of various specimens collected on Nukahiva by EDWARD JARDIN. Finding four new species native to this single small island, SCHULTZ BIPONTINUS appears to have entered upon a very careful and painstaking research into the subject of their generic affinities, finally publishing his results[1] (Flora 39:357. 1856). As regards the maintenance of a genus *Campylotheca* apart from *Bidens*, he was unreservedly against such a course. His four new species from Nukahiva and all of the Hawaiian species he referred to *Bidens*.

In my own attempts accurately to evaluate SCHULTZ BIPONTINUS' opinion, I sought four years ago to repeat his studies upon the Nukahiva species. Through the generous assistance of M. ST. AHNNE, President of the Chamber of Agriculture of Tahiti, and the careful, persistent search by his friend, M. HENRY, President of the French Alliance of Nukahiva,[2] I have been able to secure many mature achenes and herbarium specimens from the same island where JARDIN originally collected. Achenes of each kind were planted, and thus, during a period of three years, several hundred live plants have been obtained for observation. Having supplemented in this way my examination of the few herbarium specimens available, I have been able to match all of SCHULTZ BIPONTINUS' four descriptions very well. The four species (*Bidens cordifolia*, *B. polycephala*, *B. serrulata*, *B. Jardinii*) are clearly distinct in leaf characters of the older plants and in fruit characters. More-

[1] For a personal estimate, apparently unbiased and accurate, of the taxonomic ability and sagacity that SCHULTZ BIPONTINUS displayed at times, see BENTHAM, Jour. Linn. Soc. 13:340. 1873.

[2] I cannot too gratefully thank M. ST. AHNNE and M. HENRY for their great kindness shown to me during the progress of my work. Repeatedly they have assisted in procuring for me the very materials that were essential for a correct understanding of the far away Pacific Island flora.

over, none of the four is found to differ generically from the various Hawaiian species, both groups even emitting the same peculiar carrot-like odor when the leaves are bruised. There can remain no doubt, therefore, regarding the exact basis of SCHULTZ BIPONTINUS' study. Furthermore, the scholarly and critical way in which he attacked the entire subject must needs inspire a strong sense of confidence in his judgment and in the course pursued by him in equating *Campylotheca* with *Bidens*.

In 1861 ASA GRAY (Proc. Amer. Acad. 5:125–128) made the next important contribution to a knowledge of the group. GRAY had received from the Museum of Natural History in Paris several specimens collected by M. J. REMY in the Hawaiian Islands, also a number from the United States Exploring Expedition under Captain WILKES, collected in the Hawaiian Islands, Tahiti, Eimeo, and elsewhere in the Pacific. Most of these were new species. GRAY'S publication indicates that he was probably unaware of SCHULTZ BIPONTINUS' paper. Thus, for example, he inadvertently created the name *Coreopsis Macraei* for a plant already named by the latter *Bidens Campylotheca*. As, therefore, he does not seem to have read SCHULTZ BIPONTINUS' paper, it is all the more interesting and valuable to find that GRAY, too, was compelled to abandon the name *Campylotheca*. Species having the achenes wingless and the awns retrorsely barbed he described under *Bidens*. But several other species, different in having either exaristate achenes or even winged achenes, he described under *Coreopsis*. Thus he described *Bidens hawaiensis*, *B. lantanoides*, *Coreopsis mauiensis*, *C. macrocarpa*, *C. Macraei*, *C. cosmoides*, and *C. Menziesii*. GRAY'S own words at the time of describing some of these species are worthy of note. Speaking of the futility of maintaining *Campylotheca* as a separate genus, apart from *Bidens* and *Coreopsis*, he said: "Its adoption merely gives us three limitless genera unmarked by any peculiarity in habit, in the place of two artificially separated ones. . . . . Vain is the attempt to draw absolute limits where Nature luxuriates in gradations" (Proc. Amer. Acad. 5:126. 1862).

In 1888 there appeared the posthumous *Flora of the Hawaiian Islands* by WILLIAM HILLEBRAND. HILLEBRAND, from his twenty years of resident study in the Hawaiian Islands and his careful

investigations subsequently, was eminently well versed in their species. His treatment assumes almost the aspect of a monographic revision, and it is evident that he possessed much more than an ordinary knowledge of *Bidens* and related groups. His brilliancy, however, appears to have been manifested, as is so apt to occur with a local botanist, less in the excellence of his genus concept than in that of his species concept. And, even in the latter respect, his generalizations are often necessarily faulty because of the inadequacy of his material. HILLEBRAND, like GRAY, appears never to have seen SCHULTZ BIPONTINUS' paper. He discarded GRAY'S treatment, however, and adopted once again CASSINI'S name *Campylotheca*. Speaking of *Campylotheca* he says (p. 211): "The genus, as it presents itself now, stands evidently nearer to *Bidens* than to *Coreopsis*, and might be merged in the former if it were not for the winged achenes of so many species,[3] which, if admitted in the character of *Bidens*, would efface the limits between that genus and *Coreopsis*." GRAY'S Hawaiian *Bidens* is transferred by HILLEBRAND to *Campylotheca*.

This effort to break down the genus *Bidens* into smaller units is not the first of its kind. As early as 1790,[4] NECKER (Elem. Bot. 1:86–87) subdivided the genus into two new genera. For these he proposed the names *Pluridens* and *Edwarsia;* the first group to include those species with simple foliage (for example, *Bidens cernua* L.), the second to include those species with foliage dissected (for example, *B. pilosa* L. and *B. pinnata* L.). In 1794, MOENCH (Meth. 569 and 595) followed NECKER'S treatment essentially, but substituted the names *Bidens* and *Kerneria* for NECKER'S two names. Neither NECKER'S treatment nor that of MOENCH is today accepted by botanists. In 1836, DE CANDOLLE (Prodr. 5:633) described a new plant that resembled *Bidens*, but which appeared remarkable in having the ligules pistillate and fertile. DE CANDOLLE created the genus *Delucia* therefor, and his new plant he named *Delucia ostruthioides*. Later SCHULTZ BIPONTINUS (Seem. Bot. Voy. Herald 308. 1852–1857) renamed the species *Bidens ostruthioides*, and this latter name has been widely accepted

[3] Regarding the inaccuracy of this statement, cf. footnote 8.

[4] Cf. E. L. GREENE, Pittonia 4:245. 1901.

ever since.[5] In 1901, GREENE (Pittonia 4:242–270) presented the results of a study of *Bidens*. He commented upon the dissimilarity between such species as *B. cernua* L. and *B. tripartita* L. Even so radical a botanist as he, however, refrained from proposing a generic segregation of the *B. cernua* forms. Nevertheless, GREENE did segregate the aquatic *Bidens Beckii* as the type of a new genus, *Megalodonta;* and, when the peculiar achenes of this species are considered, it seems wise to accept GREENE's new genus as valid.

Strangely enough, no one appears to have tried to segregate generically the pronounced and well defined group of *Bidens* species typified by the species *Bidens reptans* (L.) G. Don.[6] These species differ from the more typical species in being climbers, and in having long flat achenes that are hispid along the two edges in such a way at times as to suggest a centipede. Again, my own *Bidens mirabilis* (BOT. GAZ. 61:496. *pl. 31.* 1916), with achenes flat, strongly constricted above into a thick neck and crowned with even 8–10 aristae, might be segregated as the type of a new genus. Similarly, the anomalous *Bidens clarendonensis* Britton, with trailing, somewhat woody stem, and thick, rhombic-ovate leaves, would be interpreted by some as representing a new genus.

Thus it is seen that, if we accept the narrow concept of *Bidens* held by CASSINI, LESSING, and HILLEBRAND, and seek to segregate the native Pacific species under the name *Campylotheca*, to be consistent we shall have to subject the entire genus *Bidens* to a process of subdivision and segregation, resulting in some six or eight genera. There are at least two good reasons for not adopting such a course. In the first place, the accuracy of such a series of interpretations is not so well established as to justify overturning almost the entire nomenclature of the genus. In the second place, the lines of demarcation among the various subordinate groups are

[5] In the herbaria *Bidens ostruthioides* is the universally used name. It is interesting to note that a closely similar form was described by KLATTE as *Bidens guatemalensis* (Bot. Jahrb. 8:44. 1887). Another related form, apparently more clearly distinct, however, was placed by BENTHAM in *Bidens* and described as *B. costaricensis* (Benth. ex Oerst., Kjoeb. Vidensk. Meddel. 94. 1852).

[6] DE CANDOLLE (Prodr. 5:599. 1836), however, did create the name *Bidens Coreopsidis* for one of these species. And, even earlier, the names *Coreopsis reptans* L., *Coreopsis incisa* Ker., etc., had been given to certain of these species, but without very serious consideration being given to their generic affiliations.

so fluctuating and inconstant that efforts to apply a binomial system of nomenclature to the many species would be rendered even much more difficult than before. I am constrained to reject, therefore, any idea of seriously interfering with the general status of *Bidens*. CASSINI's name *Campylotheca* I am compelled to discard.[7]

Having laid aside the name *Campylotheca*, there remains one further matter with which to deal. As stated previously (BOT. GAZ. 59:308. 1915), we find among the numerous species of *Bidens* and the allied genus *Coreopsis* "no absolute uniformity in even one distinctive character. However, one such character does persist to a surprising extent. It is the presence (in *Coreopsis*) or absence (in *Bidens*) of two lateral wings upon the mature achene. Among so many species from widely remote regions does this character separate two genera with different aspects that, *in cases where other criteria are absent*, it appears to offer the only logical basis of distinction." This presence or absence of achene wings was given great weight by GRAY, but in the Pacific flora the wing character is unreliable, and will lead, if absence of wings be demanded from all species of *Bidens*, to an arbitrary and unnatural arrangement. Some three or four Hawaiian forms commonly have accessory awns or barbs below the achene's apex, and either these or the principal awns frequently are decurrent along the achenial edges as a more or less thickened margin or even as a wing; or at times the awns seem unrelated to the wings. In "*Coreopsis mauiensis*" Gray, these wings are very conspicuous. The number of Hawaiian

[7] In taking this step it is reassuring to read the words of so eminent a student of the Compositae as BENTHAM. Speaking of CASSINI and his work, he stated (Jour. Linn. Soc. 13:338. 1873): "Unfortunately, however, in working out the details of the genera in the 'Dictionnaire,' he indulged in an enormous and useless multiplication of generic names, which only tended to throw the nomenclature into confusion, and cast a slur upon all his labors. Wherever he observed a slight difference in the involucre, pappus, or general aspect, or could not readily identify an imperfect specimen, an engraved figure, or a description often incorrect, he at once set it down as a new genus, and has thus, more than any other botanist of equal ability, overloaded the science with useless synonyms. So recklessly, indeed, did he give way to this mania of coining new names, that he on many occasions proposed two, or even three, for the same genus, leaving future botanists to take their choice." CASSINI did not neglect *Campylotheca* in this respect. At the very outset he proposed *Dolicotheca* as an alternative name. This latter name, however, was never adopted by LESSING, DE CANDOLLE, or others.

species that exhibit this character, however, is very small compared with the remaining Pacific species that lack it.[8] Moreover, a study of their other characters, such as odor of bruised foliage (when fresh) and shape of ligules, as well as range of distribution, shows them to be much closer to the wingless-achened *Bidens* species of the Pacific than to the wing-achened American species, *Coreopsis lanceolata* L., that must be taken as the type of the genus *Coreopsis*. It seems wise, therefore, to transfer such species directly to *Bidens* rather than leave them with *Coreopsis*, where originally placed by GRAY. We shall have even then no greater incongruity in *Bidens* than must perforce be tolerated in *Coreopsis*. Thus, for example, all authors who have dealt with the subject have retained the North American wingless-achened *Coreopsis rosea* Nutt. and *C. tinctoria* Nutt. in *Coreopsis* despite their anomalous achenes, because their other characters clearly indicated a closer affinity with *Coreopsis*. Manifestly this was the only correct course to pursue, and my own procedure is precisely comparable.

In the following list, therefore, such transfers are made. In addition, there are transferred certain other species that were described by GRAY under *Coreopsis* (where he placed them because they lacked retrorsely barbed awns; cf. BOT. GAZ. **59**:305–308. 1915), or by HILLEBRAND under *Campylotheca*. The new names are:

Bidens molokaiensis (Hillebr.), comb. nov.—*Campylotheca molokaiensis* Hillebr., Fl. Hawaiian Isls. 212. 1888.

Bidens macrocarpa (Gray), comb. nov.—*Coreopsis* (*Campylotheca*) *macrocarpa* Gray, Proc. Amer. Acad. **5**: 126. 1862.

Bidens Remyi (Hillebr.), comb. nov.[9]—*Campylotheca Remyi* Hillebr., *loc. cit.*, 212; *Coreopsis Hillebrandiana* Drake del Cast., Illustr. Fl. Ins. Mar. Pacif. 209. 1890.

[8] Cf. HILLEBRAND'S misleading words, "the winged achenes of so many species." Doubtless HILLEBRAND was recalling many specimens of a few species, and unguardedly referring to them as "so many species." Reference to his individual descriptions shows few of the species to be described as wing-achened.

[9] This species was based by HILLEBRAND upon *M. J. Remy* 287, a single specimen in Gray Herbarium. I have seen not only the type but an excellent duplicate in Paris (Herb. Mus. Hist. Nat.), also fine specimens collected by *Faurie* (Herb. Brit. Mus.), *Forbes* (Herb. Bernice Pauahi Bishop Mus.), etc. The species should not be confused with *Bidens Remyi* Drake del Cast. (Illustr. Fl. Ins. Mar. Pacif. *pl. 39.* 1888; *Coreopsis Remyi* Drake del Cast., *loc. cit.*, 210), a species founded upon *M. J. Remy* 281,

Bidens dichotoma (Hillebr.), comb. nov.—*Compylotheca dichotoma* Hillebr., *loc. cit.*, 212.

Bidens mauiensis (Gray), comb. nov.—*Coreopsis mauiensis* Gray, Proc. Amer. Acad. **5**:125. 1862; *Campylotheca mauiensis* Hillebr., *loc. cit.*, 213.

Bidens cosmoides (Gray), comb. nov.—*Coreopsis* (*Campylotheca*) *cosmoides* Gray, *loc. cit.*, 126.

Bidens Menziesii (Gray), comb. nov.—*Coreopsis* (*Campylotheca*) *Menziesii* Gray, *loc. cit.*, 127.

A most remarkable feature of the flora of the Hawaiian Islands is the large number of endemic species. For a number of years botanists have been cognizant of this pronounced degree of endemism (cf. HILLEBRAND, Fl. Hawaiian Isls. pp. xv and xxv. 1888; MACCAUGHEY, Amer. Botanist **22**:45–52. 1916; BOT. GAZ. **64**:89–114. 1917; *loc. cit.*, **66**:273–275. 1918). Furthermore, the scanty supply of Hawaiian specimens available in most herbaria often makes a proper interpretation of the various endemic forms practically impossible at the present day.

In 1917, almost in despair of being able to arrive at satisfactory opinions respecting several Hawaiian species of *Bidens*, I appealed to certain botanists resident there for aid. One of these, Professor CHARLES N. FORBES, Curator of Botany at the Bernice Pauahi Bishop Museum in Honolulu, proved able to render me assistance of the utmost value. In 1919 he placed at my complete disposal the entire *Bidens* collection of the Bishop Herbarium, also a set of duplicates (later deposited in Field Museum, Chicago). Among these were specimens not only by HILLEBRAND, MANN and BRIGHAM, and other older collectors, but also by FORBES, BRYAN, STOKES, and others of the present century. A considerable portion had

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but which clearly is a mere form of *Bidens micrantha* Gaud. A specimen of *Remy* 281 in Gray Herbarium had been erroneously determined by ASA GRAY as being *Bidens sandvicensis* Less. var. *heterophylla* Gray. Later, HILLEBRAND (*loc. cit.*, 216), having seen this sheet at Gray Herbarium and assuming GRAY'S determination to be correct, naturally equated GRAY'S *B. sandvicensis* var. *heterophylla* with *B. micrantha* Gaud. But the true *B. sandvicensis* Less. var. *heterophylla* Gray was based upon a plant in Kew Herbarium collected by BEECHEY on the Island of Oahu, and treated by HOOKER and ARNOTT as *Bidens luxurians*. This plant of BEECHEY'S is wholly distinct from *B. micrantha* Gaud. and from our *B. Remyi*.

been collected in localities never before visited by botanists. Many of the plants collected even from the better known localities were much superior in point of maturity and state of preservation to those previously collected by other botanists. No less than eleven species were found to be new. Still other species, while not new to science, were represented in such excellent or numerous forms that more elaborate descriptions and more accurate concepts were possible than when the species were first described. The descriptions, with lists of specimens examined, are presented herewith.

Bidens cervicata, sp. nov.—Glabra, supra herbacea, infra forsan suffruticosa, caule acute tetragono, ramoso, ±8 dm. alto. Folia membranacea, pinnata aut summis tripartita, petiolis adjectis 7–15 cm. longis, foliolis lanceolatis, acuminatis, serratis dentibus acribus et tenuiter mucronatis, sparsim ciliatis, 2.5–9 cm. longis et 0.8–2.8 cm. latis, petiolis tenuibus 1.5–4 cm. longis. Capitula multa, subcorymbosa, ligulata, ad anthesin 5–7 mm. alta et 1.5–1.8 cm. lata. Involucri bracteae exteriores plerumque 5, lineares, glabratae, patentes aut reflexae, 1.5–2.5 mm. longae, interioribus multo breviores. Ligulae circ. 5, flavidae, ovato-lanceolatae vel elliptico-oblongae, apice saepe profunde et acriter dentatae, 7–9 mm. longae. Achaenia tenuiter linearia, nigra, exalata, exaristata, glabra aut 1-paucis setis munita, torta, infra angustata, supra cervici-elongata, 1–1.3 cm. longa.

Specimens examined.—*C. N. Forbes* 1085 K, Waimea Drainage Basin, west side, Kauai, July 3 to August 18, 1917 (Herb. Bishop Mus., type; Herb. Field Mus., no 485172).

Bidens amplectens, sp. nov.—Herbacea supra, infra verisimiliter suffruticosa, ramosa, caule ramisque tetragonis, glabra, probabiliter 5–8 dm. alta. Folia plerumque pinnata, membranacea, petiolis adjectis 4–12.5 cm. longis et 3–7.5 cm. latis; foliolis 3–5, ovato-lanceolatis, serratis dentibus orbiculatis, ad apicem acuminatis, terminali saepe maiore, petiolis tenuibus 2–4 cm. longis. Capitula non multa, sub-solitaria in pedunculis, laxissime corymbosa, adolescentia iis Cosmi specierum non dissimilia, florescentia 6–8 mm. alta et 3–3.5 cm. lata. Involucri bracteae exteriores 5–6, valde reflexae, crassiusculae, lineari-oblanceolatae, ad apicem

subacutae et glanduloso-apiculatae, 3–6 mm. longae, quam interiores paulo breviores. Ligulae 7–8, anguste obovatae, apice obscure dentulatae, 1.5–1.8 cm. longae. Achaenia submatura nigra, plana, exalata, marginibus apiceque setulosa, exaristata aut obscurissime biaristata, circ. 8 mm. longa.

A plant of peculiar aspect, embracing habital characters of *Cosmos* and *Coreopsis* as well as of *Bidens*, hence the name *amplectens*.

SPECIMENS EXAMINED.—*C. N. Forbes* 1839 O, Kawaihapai, Waianae Range, Oahu, Hawaiian Islands, *sine tempore legendi* (Herb. Bishop Mus., type; Herb. Field Mus., no. 485361).

Bidens micranthoides, sp. nov.—Herba glabrata, infra suffruticosa, supra ramosa, ramis gracilibus, 3–5 dm. alta. Folia pinnata aut rarius ternata, petiolis adjectis 3–7 (–12.5) cm. longis et 2–5 (–8) cm. lata, foliolis ovato-lanceolatis aut raro ovatis, serratis, ad apicem plerumque acutis aut etiam longissime acuminatis, nunc membranaceis, nunc subrugoso-crassiusculis, foliolis imis raro tripartitis, petiolis tenuibis 1–5 cm. longis. Capitula supra folia exserta, laxe corymbosa, ad anthesin 5–7 mm. alta et 1.5–2.5 cm. lata. Involucri bracteae exteriores 5–7, lineares, ad apicem subobtusae, glabratae aut glanduloso-pulverulentae, 1–2.5 mm. longae, interioribus multo longioribus. Ligulae 4–6, flavae, ovato-oblanceolatae, ad apicem 2–4-dentulatae, circa 1 cm. longae. Achaenia linearia, exalata, supra et ad margines sparsim setosa, apice setoso-coronulata et biaristata aristis retrorsum hamosis aut saepe plus minusve exaristata, 7–9 mm. longa.

SPECIMENS EXAMINED.—*Captain Beechey*, Oahu (May 19–30, 1826, *fide* Hook. and Arn., Bot. Beech. Voy. p. i. 1841) (Herb. Hookeri in Herb. Kew); *C. N. Forbes* 494 K, Wailua Falls, Kauai, October 5, 1916 (Herb. Bishop Mus.; Herb. Field Mus., no. 485156); *idem* 592 K, Nonou Mountains, Kauai, October 16–17, 1916 (Herb. Bishop Mus.; Herb. Field Mus., no. 485160); *idem* 704 K, Haupu Range, above Nawiliwili Bay, Kauai, October 31, 1916 (Herb. Bishop Mus., type); *idem* 1405 O, Manoa Valley, Oahu, November 23, 1909 (Herb. Bishop Mus.; Herb. Field Mus., no. 485254); *idem* 1849 O, Waiolani Ridge, Oahu, October 27, 1913 (Herb. Bishop Mus.); *idem* 2014 O, ridge east of Kuliououiki, Oahu, November 17, 1914 (Herb. Bishop Mus.).

As the name suggests, this species resembles more or less *B. micrantha* Gaud. In some cases the resemblance in foliage is very deceiving. The preceding specimen by *Beechey* had been determined as *B. micrantha* by HOOKER and

Arnott (cf. Hook. and Arn., Bot. Beech. Voy. 86. 1841), although in this case the foliage was very distinct from that of Gaudichaud's plate for *B. micrantha*. Asa Gray, who later studied the *Beechey* plant, referred it incorrectly to *B. sandvicensis* Less. (cf. Gray, Proc. Amer. Acad. 5:128. 1862). From both *B. micrantha* and *B. sandvicensis* my species differs most noticeably in habit, being lower in stature, apparently more open in its branching, and certainly with the inflorescence much more open, the heads being variously scattered and at different levels, not so corymbose.

Bidens Stokesii, sp. nov. (pl. XII).—Supra herbacea, infra verisimiliter fruticosa, glabra, caule subtetragono, ramoso, ±6 dm. alto. Folia ternata aut 5 foliolis pinnata, membranacea, non ciliata, petiolis adjectis 4–9 cm. longa et 2.5–6 cm. lata, foliolis rhomboideo-ovatis aut lanceolatis, terminali interdum breviter acuminato, orbiculato-serratis, raro inciso-lobulatis, petiolis tenuibus 1.5–4 cm. longis. Capitula pauca, paniculato-corymbosa, tenuiter pedunculata (ad fines ramorum 10–14 cm. nudorum) pedunculis 1–5.5 cm. longis, ligulata, ad anthesin circ. 7 mm. alta et 2–2.5 cm. lata. Involucri bracteae exteriores circ. 8, lineares, glabratae aut sparsissime hispidae, apice indurato, 3–4 mm. longae, erectae aut recurvatae, interioribus longiores. Ligulae 6–7, flavidae, oblongae, apice obscure dentulatae, 7–10 mm. longae. Achaenia linearia, nigra, glabra, interdum plano-marginata sed non vere alata, saepe biaristata aristis tenuibus et obscure retrorso-hamosis, ±7 mm. longa.

Specimens examined.—*John F. G. Stokes, sine numero*, foot of plateau, southeast, Niihau, January 1912 (Herb. Bishop Mus., type).

Bidens asplenioides, sp. nov. (pl. XII).—Supra herbacea, infra verisimiliter suffruticosa, glabra, ramosa, caule subtetragono, ±4 dm. alto. Folia submembranacea, pinnata aut ternata, crenata, petiolis adjectis 6–16 cm. longa; foliolis lanceolatis aut anguste ovato-lanceolatis, non ciliatis, terminali ad apicem longe acuminato, 6–8 cm. longo, lateralibus ad apicem acutis vel subobtusis et dimidio brevioribus; petiolis tenuibus 3–7 cm. longis. Capitula multa, ligulata, ad anthesin circ. 1.5–2 cm. lata et 6–8 mm. alta, pedunculis tenuibus 1–6 cm. longis. Involucri bracteae exteriores circ. 5, lineari-spathulatae, demum reflexae, glabratae, circ. 2 mm. longae; interioribus lanceolatae, dimidio longioribus. Ligulae (mancas tantum vidi) flavae, circ. 8–10 mm. longae.

Achaenia (manca vidi) linearia, exalata, supra glabrata aut sparsim setosa, apice nuda aut biaristata, verisimiliter 5–7 mm. longa.

SPECIMENS EXAMINED.—*J. F. G. Stokes*, Kaali, Niihau, January 1912 (Herb. Bishop Mus., type).

The elongate crenate terminal leaflets offer a curious superficial resemblance in outline to the leaves or leaflets of some species of *Asplenium* (*A. pinnatifidum* Nutt. etc.). In shape of leaves, length of petioles, habit of inflorescence, number of capitula, proportionate length of exterior involucral bracts, and various other respects, this species is sharply separate from *B. Stokesii*.

Bidens valida, sp. nov.—Supra herbacea, infra verisimiliter fruticosa, glabra, caule tetragono, valido, ±7 dm. alto. Folia (exsiccata) atra supra, acriter serrata, non ciliata, petiolis adjectis 4–15 cm. longa; superiora indivisa ovata aut ovato-lanceolata, abrupte acuminata, 2–6 cm. lata; inferiora tripartita (aut interdum pinnata?—tantum unum inferius vidi), foliolis lanceolatis, acuminatis petiolis tenuibus 1–5 cm. longis. Capitula pauca, corymbosa solitaria in pedunculis subtenuibus, maiuscula, involucro ad anthesin circ. 6 mm. alto et (supra) 11 mm. lato, demum circ. 1.4 cm. alto et (supra) 1.2–3 cm. lato; pedunculis saepe bracteatis, 2–11 cm. longis. Involucri bracteae exteriores 7 aut 8, foliosae, obtuse oblongo-lanceolatae, glabrae, apice obscure induratae, demum 1.5–1.8 cm. longae et 2–3 mm. latae, interioribus longiores. Ligulae non observatae. Achaenia linearia, nigra, exalata, glabra aut sparsim setoso-hispida, apice vero exaristata, plerumque sub apicem biaristata aristis brevibus et retrorsum (1–3 setis) hispidis, 8–13 mm. longa.

SPECIMENS EXAMINED.—*C. N. Forbes* 27 K, Haupu near Lihue, Kauai, July 9, 1909 (Herb. Bishop Mus., type; Herb. Field Mus., no. 485137).

Bidens cuneata, sp. nov. (pl. XIII).—Frutex ramosus, verisimiliter 6–10 dm. altus, ramis dichotomis, tenuibus, infra foliosis, supra in pedunculos productis. Folia crassiuscula, rhomboideo-ovata, dentata (dentibus in latere singulo plerumque 3–5), ad apicem acuta, ad basim anguste aut late cuneata, petiolis adjectis 3–5 cm. longis et 1–2 cm. latis, petiolis tenuibus, 1–2 cm. longis. Capitula solitaria, ligulata, ad anthesin circ. 6 mm. alta et 2–2.5 cm. lata, pedunculis tenuibus 0.8–1.8 dm. longis. Involucri bracteae exteriores circ. 7, lineares, glabratae, glandulo-apiculatae, bracteas interiores subaequantes. Ligulae late lanceolatae, flavae, ad

apicem dentulatae, 8–11 mm. longae. Achaenia linearia, exalata, ad margines sparsissime ciliata, ad apicem ciliato-coronata, exaristata, 6–7 mm. longa.

SPECIMENS EXAMINED.—*W. A. Bryan*, Diamond Head, Oahu, in 1903 (Herb. Bishop Mus., type).

Bidens setosa, sp. nov.—Gracilis, glabra, supra herbacea, infra forsan suffruticosa, caule tetragono, ramoso, ±8 dm. alto. Folia membranacea, plus minusve ciliata, saepe sparsissime adpresso-hispida, serrata dentibus mucronatis; summa nunc indivisa, ovata aut lanceolata, ad apicem acuta vel acuminata, petiolis adjectis 3–6 cm. longa, nunc tripartita; inferiora tripartita vel pinnata foliolis ovatis vel lanceolatis, petiolis adjectis 4–7 cm. longa et 2.5–3.5 cm. lata; petiolis tenuissimis, 1–3 cm. longis. Capitula corymboso-paniculata interdum numerosa, parva, ligulata, ad anthesin 5–6 mm. alta et 1.5–2 cm. lata, pedunculis tenerrimis 1–4 cm. longis. Involucri bracteae exteriores 4–6, patentes aut reflexae, lineares, ciliatae et plus minusve pubescentes, 1.5–2.5 mm. longae, interioribus breviores. Ligulae plerumque 5, flavidae, anguste oblongo-ellipticae, apice (saepe profunde et acriter) dentatae, 5.5–8 mm. longae. Achaenia lineari-fusiformia, interiora supra anguste elongata, omnia exalata, exaristata, plerumque valde setoso-hispida setis singulis aut saepe 2–5-aggregatis, apice setoso-coronata, 7–10 mm. longa.

SPECIMENS EXAMINED.—*C. N. Forbes* 811 K, Waimea Drainage Basin, west side, Kauai, July 3 to August 18, 1917 (Herb. Bishop Mus., two type sheets; Herb. Field Mus., no. 485165).

Bidens Forbesii, sp. nov. (pl. XIV).—Herbacea supra, infra verisimiliter fruticosa, caule ramisque tetragonis, glabra, probabiliter 7–10 dm. alta. Folia inferiora magna, tripartita, petiolis adjectis 1–2.5 dm. longa et 5–15 cm. lata, foliolis lanceolatis, longe acuminatis, membranaceis, creberrime serratis dentibus acribus et longe mucronulato-inflexis, 1–1.4 dm. longis et 3.5–5 cm. latis, petiolis tenuibus 6–8 cm. longis; foliis superis minoribus, 7–10 cm. longis et 4–5 cm. latis. Capitula parva, supra folia exserta, subcorymbosa, ad anthesin 4–5 mm. alta et circ. 1.5 cm. lata. Involucri bracteae exteriores circ. 3–4, anguste lineares, ad apicem acutae, glanduloso-pulverulentae aut fere glabratae, patentes aut

reflexae, circ. 1.5 mm. longae, interioribus paulo longioribus. Ligulae circ. 5, flavidae, anguste oblongo-obovatae, apice valde acriterque 2-dentatae, 6–8 mm. longae. Unum achaenium maturum visum nigrum, valde arcuatum et tortum, glabrum, exalatum, exaristatum, circ. 1 cm. longum; achaeniis immaturissimis biaristatis, aristis retrorsum 1–2-hamosis.

SPECIMENS EXAMINED.—*C. N. Forbes* 82 K, Waioli Valley, Kauai, July 23, 1909 (Herb. Bishop Mus., two type sheets).

Bidens waianensis, sp. nov.—Frutex glaber, supra ramosus, verisimiliter 5–8 dm. altus. Folia gracilia, pinnata aut plus minusve bipinnata, petiolis adjectis 4–12 cm. longa et 3–6 cm. lata, foliolis primariis lanceolatis serratis acuminatis aut iterum pinnatis lobis ultimis linearibus integris ad apicem acriter mucronatis, petiolis tenuissime 2–4 cm. longis. Capitula multa, corymbosa aut corymboso-paniculata, ad anthesin circ. 6 mm. alta et 1.5–2 cm. lata, breviter supra folia exserta, floribus 15–25. Involucri bracteae exteriores circ. 6, lineares, glabratae aut sparsim glanduloso-pulverulentae, ad apicem subacutae, 1–2 mm. longae, quam bracteae interiores dimidio breviores. Ligulae circ. 5, flavidae, oblongo-oblanceolatae, ad apicem obtusae, circ. 1 cm. longae. Achaenia nigra, valde torta, glabra aut versus apicem remote setosa, exalata matura exaristata et 6–10 mm. longa.

SPECIMENS EXAMINED.—*C. N. Forbes* 2023 O, Kolekole Pass, Waianae Range, Oahu, February 1 and 2, 1915 (Herb. Field Mus., no. 485291, type; Herb. Bishop Mus., a form with leaflets much broader than in the type specimen); U.S. S. Pacif. Expl. Exped. (under *Captain Wilkes*), Kaala Mountains, Waianae Range, Oahu, 1838–1842 (Herb. Bishop Mus.; Herb. New York Bot. Gard., two sheets); *J. F. G. Stokes*, Kolekole Pass, Waianae Range, Oahu, in 1915 (Herb. Bishop Mus.).

ASA GRAY had determined the specimens collected under Captain WILKES as being *Bidens micrantha* Gaud. (*Coreopsis micrantha* Gray). Later, in discussing *Bidens micrantha* (Proc. Amer. Acad. 5:127. 1862), he said: "Sandwich Islands, especially Oahu. Variable in the foliage, which is commonly more dissected than in GAUDICHAUD'S figure." Clearly GRAY had in mind the *Wilkes* plants, collected in the Waianae Range on Oahu. A study of the more recent specimens by FORBES and by STOKES, collected in the same immediate locality, shows the identical peculiarities of foliage. Furthermore, the floral and achenial characters are seen to be very distinct from those of the more widely distributed *Bidens micrantha*, which occurs not only on Oahu but also on Hawaii, Maui, and Lanai.

Bidens torta, sp. nov.—Fruticosa, glabra, caule non crasso ± 5 dm. alta. Folia tripartita, membranacea, serrata, ciliata, tenerrime petiolata, petiolis adjectis 7–16 cm. longa et 2.5–10 cm. lata, foliolis acuminatis, terminali multo maiore, oblongo-lanceolato, lateralibus sessilibus aut breviter petiolulatis, ovato-lanceolatis, petiolis 1–4 cm. longis. Capitula numerosa, laxe paniculata, mediocria, ligulata, ad anthesin circ. 5 mm. alta et 1.7 mm. lata. Involucri bracteae exteriores circ. 5, tenuiter lineares, glanduloso-pubescentes, 1.5–2.5 mm. longae, interioribus paulo breviores. Ligulae circ. 5, oblongo-oblanceolatae, flavae, ad apicem plus minusve dentulatae, circ. 7 mm. longae. Achaenia tenuiter linearia, nigra, maxime torta, glabra, corpore 9–13 mm. longa ad apicem calva aut obscure 1–2-aristata aristis glabris brevissimis (0.1–0.3 mm. longis).

SPECIMENS EXAMINED.—*C. N. Forbes* 2092 O, Kawailoa, Oahu, March 2–5, 1915 (Herb. Bishop Mus., type; Herb. Field Mus., no. 485294).

The leaves of this species appear to have rather large leaflets in proportion to the thickness of the petiole. The terminal leaflet becomes 1 dm. long and 4.4 cm. wide. The branches of the inflorescence are slender and widely diverging. The leaves and inflorescence combine to give a striking superficial resemblance to certain Central American specimens of *B. squarrosa* H. B. K. The achenes surpass those of nearly all other species in the amount of twisting. The twisting commences early, in the young achene, and the mature achenes are frequently twisted through four or five complete revolutions.

BIDENS GRACILIS Nutt., Trans. Amer. Phil. Soc. Ser. II. **7**:368. 1841; *Campylotheca gracilis* Walp., Rept. Bot. Syst. **2**:618. 1843.—No described species of *Bidens* has been left heretofore in greater obscurity than *B. gracilis*. From the time of NUTTALL's original description, no botanist appears to have given it serious attention. In 1843 WALPER categorically transferred the species, along with two others described by NUTTALL, to *Campylotheca*. In 1862 GRAY (Proc. Amer. Acad. **5**:128) referred it, along with *Bidens mutica* Nutt., to *B. sandvicensis* Less. NUTTALL's types of *B. gracilis* and *B. mutica* are still extant in a state of excellent preservation (Herb. Brit. Mus.). The type of *B. gracilis* is clearly distinct from that of *B. mutica*. It is distinct also from the specimens that I assume to be of the type collection of *B. sandvicensis* Less. by CHAMISSO from Oahu (for example, *distrib. Acad. Petropol. in Hb. Kew, ex Hb. Hookeri*). In 1888 HILLEBRAND doubtfully referred

the species to a variety of *Bidens macrocarpa*, but NUTTALL'S type is not even remotely matched by the type material (Herb. U.S. Nat. Mus.; Herb. N.Y. Bot. Gard.; Herb. Gray) of *B. macrocarpa*. It is, however, the same as *Mann* and *Brigham* 98, distributed to various herbaria as *B. hawaiensis*. *B. hawaiensis* is a much coarser plant and differs in many characters from *B. gracilis*. From all other species of the Hawaiian Islands *B. gracilis* is sharply distinct. From the several specimens studied, I have drawn up the following amplified description:

BIDENS GRACILIS Nutt., descript. amplificat. (pl. XIII).—Frutex gracilis, glabra, ramosa ramis rubescentibus, verisimiliter 6–9 dm. alta. Folia plerumque serrata aut etiam laciniato-dentata, acuminata; nunc indivisa et ovata aut lanceolata, petiolis adjectis 3–7.5 cm. longa et 1–2 cm. lata; nunc tripartita, foliolis lanceolatis, foliolo terminali 4–5 cm. longo et 1–1.5 cm. lato, lateralibus dimidio minora; petiolis tenuibus, 1–2 cm. longis. Capitula parva, paniculata paniculis trichotomis, ligulata, ad anthesin 6–7 mm. alta et circ. 1 cm. lata, pedunculis gracilibus 0.5–2.5 cm. longis. Involucri bracteae exteriores lineares, patulae, supra subglandulosae, interioribus adpressis fere dimidio breviores. Ligulae circ. 5, lanceolatae, flavae, ad apicem dentatae, 3–6 mm. longae. Achaenia torta, linearia, corpore ± 8 mm. longa, facie et marginibus glabra aut sparsissime hispida, apice setuloso-ciliata, nunc brevissime biaristata aristis 0.3–0.8 mm. longis et glabris aut versus apicem retrorsum hispidulis, nunc uniaristata aut etiam exaristata, saepe omnibus tribus formis in eodem capitulo.

SPECIMENS EXAMINED.—*Thos. Nuttall*, Oahu (Herb. Brit. Mus., type); *Mann* and *Brigham* 98, Oahu (Herb. Bishop Mus.; Herb. Cornell Univ.; Herb. Field Mus.); *C. N. Forbes* 1184 O, Moanalua Valley, Oahu, March 9, 1909 (Herb. Bishop Mus.).

BIDENS MICRANTHA Gaud. Voy. Freycinet Bot. 464. *pl. 85*. 1826–1829.—*Campylotheca micrantha* Cass. Dict. Sci. **51**:475. 1827; *Coreopsis micrantha* Gray, Proc. Amer. Acad. **5**:127. 1861; *Bidens Remyi* Drake del Cast. Illustr. Fl. Ins. Mar. Pacif. *pl. 39*. 1888; *Coreopsis Remyi* Drake del Cast., *loc. cit.*, 210.—The identity of *Bidens micrantha* Gaud. has long been a matter of conjecture with most authors. Many appear to have assumed that GAUDI-

CHAUD's original plate was only a crude representation, and that hence the delineation of foliage, etc., given there must not be interpreted very literally. Consequently, various other species have been referred arbitrarily to *B. micrantha* to such an extent that references in literature to *B. micrantha* Gaud. are almost entirely untrustworthy. In studying the recent collections from the Hawaiian Islands, I was impressed with the resemblance of a certain plant (*G. C. Munro* 602, see later) to GAUDICHAUD'S illustration. The leaves possessed the same peculiar outlines as in the drawing. A careful study of the plant showed that it was positively the true *B. micrantha*. Several other plants that, while varying in various minor details from this plant, were seen to belong nevertheless with it specifically, were then assembled. From this small group of specimens I have been able to draw up the following amplified description.

BIDENS MICRANTHA Gaud., descript. amplificat.—Frutex glabra, caule plus minusve rubido, 6–9 dm. alta. Folia gracilia, crassiuscula, irregulariter 3–5-foliolata aut summa simplicia, petiolis adjectis 4–13 cm. longis, foliolis anguste lanceolatis, acuminatis, utroque latere paucis dentulis ad medium serrato, 2–5 cm. longis et 4–12 mm. latis, petiolis 1.5–5 cm. longis. Capitula numerosa, paniculata aut corymbosa, ligulata, ad anthesin 4–6 mm. alta et 1.5–2 cm. lata, pedicellis tenerrimis 1–2.5 cm. longis. Involucri bracteae exteriores lineares, resino-pubescentes aut glabratae, minimae (circ. 1.5 mm. longae), bracteis interioribus multo minora. Ligulae 3–5, anguste oblongae, flavae, saepe ad apicem obscure dentatae, circ. 1 cm. longae. Achaenia linearia, nigra, compressa, recta vel torta, facie et marginibus plerumque glabra, 7–10 mm. longa, apice nunc exaristata et setosa, nunc breviter biaristata aut etiam (marginibus excurrentibus sub apicem) irregulariter quadriaristata, aristis glabris aut retrorsum hispidulis.

SPECIMENS EXAMINED.—*M. J. Remy* 281, Hawaii, 1851–1855 (Herb. Gray; Herb. Mus. Hist. Nat. Paris; type material of *Bidens Remyi* Drake del Cast *non nobis; Coreopsis Remyi* Drake del Cast.); *C. N. Forbes* 14 H., Puuwaawaa, Hawaii, June 8–14, 1911 (Herb. Bishop Mus.); *idem* 326 Mo., ridge and foot of Lahainaluna Valley, Maui, February 1913 (Herb. Bishop Mus.); *G. C. Munro* 602, ridge to Puu Kukui, Maui, September 26, 1916 (Herb. Bishop Mus.; one of the achenes was 3-awned!); *idem* 122, Waiapaa, Lanai, September 26, 1913

(Herb. Bishop Mus.; form very close to GAUDICHAUD's original plate); *C. N. Forbes*, Kaala Mountains, Makaha Valley, Oahu, February 12–17, 1909 (Herb. Bishop Mus.; Herb. Field Mus., no. 485330; a form somewhat atypic as to foliage); *Hillebrand* and *Lydgate*, Kula, Maui (Herb. Bishop Mus.).

BIDENS MACROCARPA (Gray) Sherff.—*Coreopsis* (*Campylotheca*) macrocarpa Gray, Proc. Amer. Acad. 5:126. 1862.

BIDENS MACROCARPA, descript. amplificat.—Fruticosa, erecta, glabra (1–1.6 m. alta fide Hillebr. Fl. Haw. 214. 1888). Folia subcrassa, ternata aut pinnata aut summa saepe maximam partem simplicia, petiolo adjecto 0.5–2.2 dm. longa; foliolis (3–5) ovatis aut ovato-lanceolatis, cuspidatis, acriter et saepe creberrime serratis (dentibus interdum valde inflexis), lateralibus 2–6 cm. longis et 1–2 cm. latis, terminali maiore et saepius acuminato, petiolulis lateralium plerumque 2–15 mm. longis; petiolis tenuibus, 2–10 cm. longis. Inflorescentia laxa, aperta, foliolis linearibus vestita, folia superans; capitulis non numerosis, non minutis, ligulatis, ad anthesin 7–8 mm. altis et circ. 3 cm. latis. Involucri bracteae subaequales, exteriores (5–7) crassae, late lineares, glabrae, circ. 6 mm. longae. Ligulae (5–7) flavae, trifida, 1–1.6 cm. longae; disci floribus 15–20. Achaenia pro capitulo magna, late linearia, striata, glaberrima aut marginibus et apice setulosa, erecta aut subtorta, 1.2–2 cm. longa, exalata aut anguste alata, alis in duo dentes aut aristas sub achaeni corporis apicem productis; aristis remotissime et minutissime, antrorsum et retrorsum barbatis, raro glabratis.

SPECIMENS EXAMINED.—*Capt. Wilkes*, U.S. S. Pacif. Expl. Exped., 1838–1842, Oahu (Herb. U.S. Nat. Mus., type; Herb. N.Y. Bot. Gard.; Herb. Gray); *Hillebrand* and *Lydgate*, Konahuanui, Oahu, Hawaiian Islands, October 1872 (Herb. Bishop Mus.); *A. A. Heller* 2901, on and near the summit of Konahuanui, Oahu, November 2, 1895 (Herb. Mo. Bot. Gard.; Herb. N.Y. Bot. Gard.; Herb. U.S. Nat. Mus.); *C. N. Forbes* 2221 O, Wahiawa-Kahana trail, Oahu, August 17–20, 1915 (Herb. Bishop Mus.); *idem*, Palolo Valley ridges, Oahu, December 17, 1908 (Herb. Bishop Mus.); *idem* 2313 O, ridge and foot, Kalihi Valley, Oahu, March 9, 1916 (Herb. Bishop Mus.); *idem*, Lanihuli Trail, Oahu, October 14, 1908 (Herb. Bishop Mus.); *idem* Koolauloa Mountains between Punaluu and Kaipapau, Oahu, May 3–8, 1909 (Herb. Bishop Mus.); *idem* 2553 O, Manoa Ridge, Oahu, March 17, 1919 (Herb. Bishop Mus.).

A distinguishing character of this species is the appearance of the large fruiting heads. The achenes become elongate, wide, thickish, and usually very glabrous. In no other species is the tendency to have awns placed below the achene's top (that is, upon the margins and more or less decurrent with the achene edge or wing) more pronounced than here.

SHERFF on BIDENS

SHERFF on BIDENS

SHERFF on BIDENS

Bidens linearifolia (O. and H.), comb. nov.—*Coreopsis linearifolia* Oliver and Hiern, Fl. Afr. Trop. **3**:390. 1877; *Bidens Schweinfurthii* Sherff, Bot. Gaz. **59**:309. 1915.—In 1915, on finding it necessary to transfer this species from *Coreopsis* to *Bidens*, I purposely created the new name *Bidens Schweinfurthii* "to avoid any possible confusion with *Bidens linearifolia* Schz. Bip." (Bot. Gaz. **59**:309). This appeared to me to be much the most desirable procedure. Nevertheless, in view of the fact that *Bidens linearifolia* Schz. Bip. is not a true *Bidens*, but is rather a species of *Cosmos* (*C. linearifolius* Hemsley, Biol. Centr. Amer. **2**:200. 1881), the International Rules require the retention of Oliver and Hiern's trivial name.

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EXPLANATION OF PLATES XI–XIV

PLATE XI

Bidens exigua (*a–f'*) and *B. duranginensis* (*g–m'*): *a*, an entire plant ×0.64; *a'*, fruiting head ×0.64; *b*, *b'*, exterior involucral bracts ×5; *c*, interior involucral bract ×5; *d*, palea ×5; *e*, disk floret ×5; *f*, *f'*, outer and inner achenes ×5; *g*, branch and portion of main stem ×0.64; *h*, exterior involucral bract ×5; *i*, interior involucral bract ×5; *j*, ligulate floret ×5; *k*, palea ×5; *l*, disk floret ×5; *m*, *m'*, outer and inner achenes ×5; *a–f'*, from type of *B. exigua* in U.S. Nat. Herb.; *g–m'*, from type of *B. duranginensis* in Herb. Gray.

PLATE XII

Bidens asplenioides (*a–f*) and *B. Stokesii* (*g–o*): *a*, branch ×0.61; *b*, exterior involucral bract ×6; *c*, interior involucral bract ×6; *d*, ovary ×6; *e* and *f*, fragments of mature achenes ×6; *g–i*, branch and additional leaves ×0.61; *j*, exterior involucral bract ×6; *k*, interior involucral bract ×6; *l*, ligulate floret ×6; *m*, palea ×6; *n*, disk floret ×6; *o*, achene ×6; *a–f*, from type of *B. asplenioides*, *g–o*, from type of *B. Stokesii*, both in Herb. Bishop Mus.

PLATE XIII

Bidens gracilis (*a–i*) and *B. cuneata* (*j–p*): *a*, branch ×0.71; *b*, leaf ×0.71; *c*, exterior involucral bract ×7; *d*, interior involucral bract ×7; *e*, ligulate floret ×7; *f*, palea ×7; *g*, disk floret ×7; *h* and *i*, achenes ×7; *j*, branch and two old peduncles, apparently of previous season's growth ×0.71; *k*, exterior involucral bract ×5; *l*, interior involucral bract ×5; *m*, ligulate floret ×5; *n*, palea ×5; *o*, disk floret ×5; *p*, achene ×5; *a*, *c–i*, from *Mann* and *Brigham* 98, Herb. Bishop Mus.; *b*, illustrating trifoliate leaf, from *Forbes* 1184 O. Herb. Bishop Mus.; *j–p*, from type of *B. cuneata*, Herb. Bishop Mus.

PLATE XIV

Bidens Forbesii: *a*, upper portion of plant ×0.61; *b*, leaf from sterile branch ×0.61; *c*, exterior involucral bract ×6; *d*, interior involucral bract ×6; *e*, ligulate floret ×6; *f*, palea ×6; *g*, disk floret ×6; *h*, very young achene ×6; *i*, mature achene ×6; *a*, *c–i*, from first type sheet, *b*, from second type sheet in Herb. Bishop Mus.

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STEM ANATOMY OF DIOON SPINULOSUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 270

LaDema M. Langdon

(WITH PLATES XV–XVII AND FOUR FIGURES)

Introduction

Investigations dealing with the minute anatomical structure of the adult cycad stem have become very numerous and more or less thorough for all of the genera and many of the species, with the exception of the great Mexican representative, *Dioon spinulosum*. This unique and interesting species was first but inadequately described by Eichler (6) in 1883, and by Dyer (5) in 1885. The first extensive account of its general field characters, size, external structure, and distribution was by Chamberlain (1) in 1909. A later article (2) by the same author gives a full and careful description of the macroscopic structure of adult stems of *Dioon spinulosum*, *D. edule*, *Ceratozamia mexicana*, and *Zamia floridana*, particular attention being given to *D. spinulosum*. Special study is made of the growth rings, reported here for the first time in cycads, and of the medullary bundles which constitute the vascular system of the cones, and which are called cone domes, because of the domelike arrangement of these strands with the peduncle of the cone at their apex. The histological characters of the trunk, its growth rings, the thick-walled fibers of the phloem, and the structure of the xylem elements the author considers remarkably similar to the corresponding structures of *Cycadeoidea*.

The embryo and seedling of *D. spinulosum* have been studied recently by Sister Helen Angela (4), and found in the arrangement and orientation of the vascular strands in the cotyledons, hypocotyl, stem, and leaves to differ in no marked degree from the usual cycad arrangement. Features particularly worthy of note in connection with the girdling habit, as this investigator has traced it from macerated seedling stems, is that each leaf is supplied with five strands arising from cauline bundles situated in different parts

of the stem, and further, that these girdling strands are horizontal from the beginning and continue so throughout their whole extent.

The investigation here described was undertaken with a view to supplementing CHAMBERLAIN'S general account of the histological structure of the adult stem of *D. spinulosum*, especially by a careful study of the broad foliar rays or leaf gaps with their included traces, a phase of cycadean anatomy only slightly touched upon by earlier investigators. As the work has progressed, its scope has been extended to include the general course and organization of the foliar strands in the cortical part of both adult and seedling stems.

Material and methods

The adult wood and abundant material of two- and three-year-old seedlings, which furnish the basis for this study, were secured by CHAMBERLAIN from the Hacienda de Joliet near Tierra Blanca, Mexico. The ten-year-old seedlings were from seeds procured in the same locality but germinated and grown in the botanical greenhouse of the University of Chicago.

Preparation of all material of the adult stem for sectioning was in the main as follows. Narrow, wedge-shaped sections extending radially from pith to cortex were cut from both the upper and lower portions of a trunk 18 ft. in height, care being taken that each included two or more of the large medullary rays. These sections were then cut into blocks about 1 cm. square, some slightly larger, and kept in series.

The various stages involved in the preparation of these blocks for sectioning, namely, demineralization of the woody tissues through the use of hydrofluoric acid, followed by a thorough washing of the material in running water to free it from all traces of the reagent, transference to various grades of alcohol and xylol, and finally imbedding in paraffin, have been discussed in a previous paper (8). Special care had to be taken in imbedding, the best results being obtained when the blocks were carried through the process of infiltration with paraffin from 48 hours to 3 days. After this they could easily be cut with a sliding microtome, and a complete series obtained by removing each section, as cut, from the knife and placing it directly upon the slide. Staining was with

safranin and gentian violet, or safranin and "licht grün," the latter combination proving the more satisfactory.

The greater part of the study of the girdling habit in the seedlings was made from cleared material. Entire sections comprising stem cylinder and cortex, in blocks 0.5×1 inch, were cleared so perfectly that it was possible, with the aid of a strong artificial light, to look through such a section and see the vascular strands clearly outlined in the cortex.

In the case of the two- and three-year-old seedlings, the method followed consisted in severing the long taproot from the stem just below the region of the cotyledonary plate and cutting off the long terminal leaves, leaving only the leaf bases and a small part of the petiole. The scale leaves were then carefully trimmed from two sides of the stem, and one clean longitudinal cut made through the entire stem from apex to base. After the transference of these half-sections to 50 per cent alcohol (each seedling being kept in a separate receptacle), the process was substantially the same as that for the paraffin method, that is, up to the pure xylol stage. At this point the material was subjected to vacuum treatment in order to free the tissues, as far as possible, of any air or gases they might contain. As a final clearing agent a mixture of xylol and carbon disulphide was used; the CS_2, having a higher refractive index, rendered the material more transparent than pure xylol.

Adult stem

With the single exception of the Australian *Macrozamia Hopei*, *Dioon spinulosum* Dyer is the tallest of all cycads, ranging 10–30 ft. in height, with occasional specimens reaching 40 and even 50 ft. The particular specimen from which this study was made was about 18 ft. tall and possibly 100 years old. The width of the woody zone from pith to cortex averages 0.5–0.75 inch in the upper part of the trunk and 3.5–4 inches in the lower trunk.

Structure of xylem.—The adult stele of *D. spinulosum* is endarch, and its compact woody cylinder consists chiefly of longitudinal tracheidal elements and radial parenchyma. From the pith to the cortical part of the stem the length of the tracheids averages as follows: scalariform metaxylem tracheids 4–4.2 mm.,

first pitted tracheids 5–6.5 mm., tracheids in the vicinity of the cambium 7–9.8 mm.

The protoxylem elements are of the reticulate and scalariform types, and in passing from the metaxylem to the first formed elements of the secondary wood all transitional stages occur in the reduction of the scalariform structure into imperfectly formed, multiseriate, bordered pits.

While the majority of the tracheids of the secondary xylem exhibit on their radial walls the multiseriate type of pitting so characteristic of this wood, many of the tracheidal elements, especially those constituting the secondarily formed wood in the upper trunk, have their radial walls covered with small bordered pits of a very irregular arrangement.

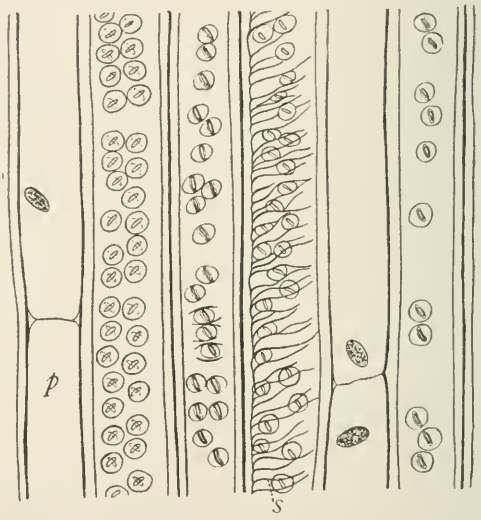

FIG. 1.—Radial longitudinal section of tracheids from lower portion of adult trunk: *s*, tertiary spiral thickenings of tracheid walls; *p*, wood parenchyma; ×225.

In the wood of the lower trunk tertiary spiral thickenings of the tracheid walls were observed occurring in the first few rings of growth and also in the older wood (fig. 1). These spirals are not common to all the tracheids, but are generally sporadic in their appearance and may be quite inconspicuous. In some cases, however, they are characterized by considerable prominence, and are so compact as to suggest a reticulate rather than a spiral formation.

In addition to the lignified elements of the wood there are narrow elongated cells with transverse walls, the longitudinal storage parenchyma. These cells, like those of the radially disposed

bands of parenchyma, are usually well filled with starch and occasional calcium oxalate crystals.

MEDULLARY RAYS.—The medullary rays are of three types, namely, narrow uniseriate rays, a single layer of cells wide and several cells deep; multiseriate rays, two to several cells in width at their widest point and of variable longitudinal extent; and broad foliar rays or leaf gaps, which in tangential view resemble the aggregate ray of *Quercus*. The last are distributed at fairly equal intervals throughout the woody cylinder and always extend from the pith to the cortex. They are further characterized by the presence of at least one mucilage duct and one leaf trace bundle situated in the lower central part of the gap (fig. 6). A few isolated cases occur where two ducts and even two traces may be seen in a single foliar ray.

Course and structure of leaf trace in gap

The course of the leaf trace through the parenchymatous gaps or foliar rays from pith to phloem is approximately level, except for a slight downward curve of the strands due to their manner of formation. The bundle is endarch throughout its course in the gap and through the phloem, the xylem of the bundle usually uppermost and just beneath the duct, with no change in orientation until the bundle reaches the cortex, where it is continuous with one of the oblique cortical strands.

The manner of connection of this foliar trace with the primary and secondary wood of the main stele is one of the most striking and interesting features of the wood. Within a short distance of the pith the strands of the trace curve downward, the primary and secondary elements uniting with like elements of the main cylinder. On the interior vertical face of the wood at the point of union, and continuing up through the gap, always on the upper side of the trace (figs. 3–5) where the primary vessels of the trace would naturally appear, are peculiar tracheidal elements, curiously reticulated, in some cases forming continuous vessels, in other cases merely isolated patches of lignified tissue. These irregular fibrous elements are best illustrated in fig. 2, where a longitudinal section of the upper portion of the trace appears in a transverse section of the wood.

Worsdell (12) also calls attention to the occurrence of peculiar irregular tracheids resembling "transfusion-tissue" on the interior vertical face of the wood and accompanying the bundles of the large medullary rays of *Macrozamia Fraseri*, and also found among

Fig. 2.—Transverse section of mature wood, showing foliar ray in center: *T*, longitudinal section through upper part of leaf trace; ×85.

the parenchyma cells between successive vascular rings in *Encephalartos* (13). These, however, are of a pitted type rather than of the irregularly reticulated and scalariform types characteristic of *D. spinulosum*.

The mode of connection of this trace with the secondary fibrovascular structures of the stem is as follows. Figs. 3, 4, and 5 are

radial longitudinal sections of the large medullary ray with its included foliar strand. A careful study of a series of such radial sections through the trace has made it apparent that this connection is by means of long, irregularly shaped scalariform tracheids which are the real tracheids constituting the trace, and not mere con-

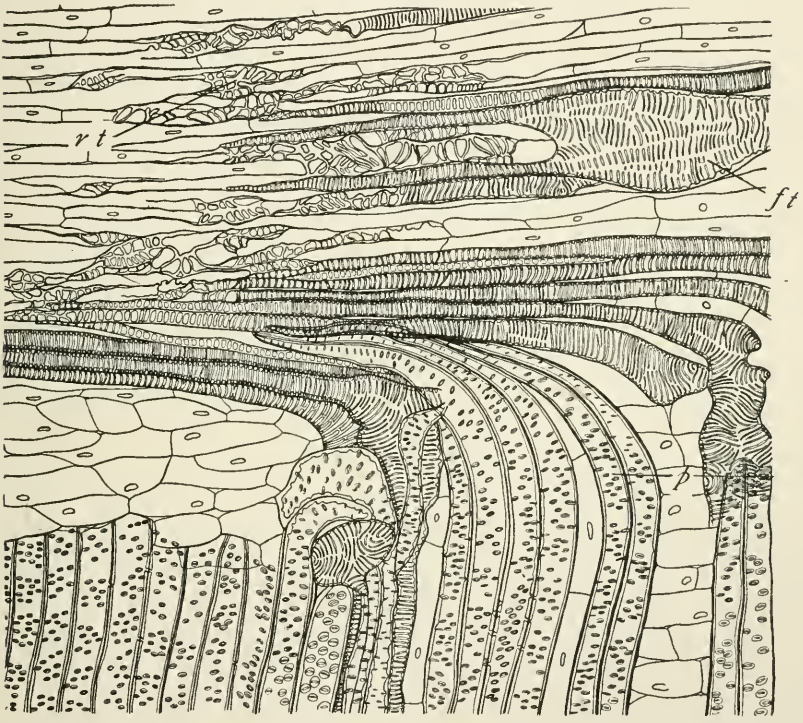

FIG. 3.—Radial longitudinal section of portion of large medullary ray with included foliar strand: *ft*, peculiar tracheidal formation apparently resulting from fusion of two or more tracheids; *rt*, irregularly reticulated elements; ×90.

necting elements, as generally believed. These vessels may be entirely scalariform in structure, or may change gradually from a reticulate type at their tapering ends to decidedly scalariform throughout the greater part of their horizontal and vertical extent. As they extend horizontally through the gap they are arranged in order of formation, one vessel beneath the other (figs. 4, 5), the

lowest and last formed continuing a little farther in the direction of the cortex than the one preceding. In this way they constitute a perfectly continuous conducting system for solutions passing out to the cortical strands. These tracheids are especially numerous a short distance behind the pith, just beyond the primary xylem, but passing on through the gap they appear to become grouped (fig. 5), occurring at intervals with great masses of phloem and parenchyma separating the basal parts of each group. Whether this grouping is associated with a seasonal increase in the length of the trace, corresponding to the radial increase and growth rings of the main stele, has not been determined. In addition to the reticulate and scalariform elements constituting these traces, the regular pitted tracheids of the secondary xylem, usually at the extreme lateral borders of the gap, are often diverted to one side into a direction more or less parallel to that of the trace, as shown in figs. 3 and 5.

Worsdell (12) has described in the case of *Macrozamia Fraseri* a somewhat similar connection between the fibrous strands in the large medullary rays and the fibrovascular elements of the main stele. He states that "a characteristic feature of the radial section of the wood is the large number of outbending strands of tracheids which, passing through the medullary rays, are continuous with the girdle leaf trace of the cortex." It is worthy of note, however, that these strands of outbending tracheids, or rather inbending in the sense that they are apparently diverted from the direction of the other secondary xylem elements toward that of the rays, are of the pitted type throughout their length, thus homologous with the pitted elements described in the preceding paragraph.

The peculiar down-curving growth of the scalariform tracheids constituting the foliar strands in the large medullary rays of *D. spinulosum* is another interesting illustration of the much discussed phenomenon of gliding growth. Vertically these conducting elements may extend merely to the lower borders of the gap and terminate in the irregular bulbous formations illustrated in fig. 4*B*, or they may become inserted for a considerable depth between the perpendicular fibrous elements of the main stele (fig. 5*sc*). In their horizontal extent these tracheids may be and probably are

the product of cambial activity, but in their vertical enlargement and elongation it seems probable that these lignified elements have simply been stretched out into their curious bending shapes by the growth of the adjoining living parenchyma tissue. The close scalariform markings on these vessels, in some cases approaching almost a pitted character, would indicate that this growth or elongation has taken place gradually, keeping pace with the longitudinal expansion of the gap.

It is also evident that the basal portions of many of these vessels have their origin in quite a different manner. The character of the pitting indicates that there has been a gradual lignification of the ordinary parenchyma cells (fig. 4 *B*), and a subsequent fusion by the breaking down and reabsorption of the partition walls. The formation of the peculiar curved and bulbous-like bases of many of these tracheids, where they come in contact with the perpendicular elements of the secondary wood, is shown in this way.

Course of leaf traces in cortex

The course of the fibrovascular bundles in the cortex, complicated by the well known habit of girdling, was first described by KARSTEN (7) in *Zamia muricata*, in 1856, later, in 1861, by METTENIUS (10), and in more recent articles quite fully by THIESSEN and Sister HELEN ANGELA in seedlings of *Ceratozamia* (3), *Dioon edule* (11), and *D. spinulosum* (4).

A brief statement of the girdling situation in the embryo and seedling of *Dioon edule*, as described by THIESSEN, is approximately as follows. For each leaf or scale leaf there are four distinct strands leaving the vascular cylinder. Two of these leave on the same side as the leaf for which they are destined, and pursue a direct course through the cortex to the central part of the petiole without branching; while the other two strands leave the cylinder approximately on the opposite side, describe a wide curve around it, and finally enter the dorsal part of the leaf petiole, where they branch repeatedly.

Sister HELEN ANGELA (4) has described a similar situation and arrangement of the cortical traces in the seedlings of *Dioon spinulosum*. Both authors agree that there are 4 or 5 strands leaving

the main cylinder for each leaf, each one of these strands describing a separate arc to the point where it enters a leaf base. Furthermore, all girdles are reported as being horizontal throughout their whole extent. It is obvious, therefore, that the phenomenon of girdling, as I have been able to trace it very distinctly and definitely in cleared specimens of two-, three-, and ten-year-old seedlings of *D. spinulosum*, differs in many respects from these earlier accounts. Thus for each leaf or scale leaf 7–9 strands, the number varying with the size of the sheathing leaf base, separate from the vascular cylinder. Two of these (fig. 8 *e*, e^1) leave the cylinder on the same side as the leaf for which they are destined and take an upward, oblique course for some distance, finally passing out more or less directly through the cortex into the ventral part of the petiole.

Two other traces (fig. 9 *a*, a^1) leave the main stele at closely approximated points on the side opposite the leaf for which they are destined and pursue an upward, rather oblique course for some distance. Then, curving one in either direction, they take a horizontal course, describing wide arcs through the cortex and sheathing leaf base, finally entering the dorsal or adaxial part of the petiole, where they undergo a complicated system of branching. The rest of the traces destined for this leaf (fig. 9 *b*, b^1, *c*, c^1, etc.) leave the main stele at intermediate points and assume, like traces (*a*, a^1), an upward, vertical direction, finally anastomosing with the two horizontal strands as they encircle the cortex. It is also noteworthy that each of these lateral oblique traces leaves the stem cylinder at a point slightly higher than the one preceding, so that the entire course of the two girdles is gradually and spirally ascending to the point where they enter the central part of the leaf base. Frequently a single bundle (fig. 8 *a*), separating from the vascular cylinder on the side opposite the leaf for which it is destined, may divide soon after leaving the central cylinder, the two horizontal branches swinging to right and left in wide curves through the cortex and the sheathing base of the leaf, and gradually anastomosing with the rest of the traces destined for that leaf. The character of the branching of these two main strands after entering the adaxial part of the petiole is so clearly illustrated in fig. 10 that any further discussion of this point is unnecessary.

At the very tip of the stem the traces of the youngest leaves ascend in an almost perpendicular direction about the region of the so-called potential vascular tissue to the point where they connect with the horizontal bundles. At this stage (fig. 10) all of the girdling strands lie in substantially the same plane, the pair associated with the youngest leaf describing slightly smaller arcs than those of the older leaves of the same crown. As internal radial growth and the appearance of new leaves crowd the older parts farther and farther away from their original terminal position, however, the lateral foliar traces become less vertical and more oblique. With this radial and longitudinal expansion of the stem is also associated a lengthening of the horizontal girdling strands, and consequently a widening of the intervals and the arcs between each lateral connecting trace.

These leaf traces are always endarch and collateral as they leave the stem cylinder, and also during their passage vertically and horizontally through the cortex to a point well up in the leaf base. They are so orientated that the xylem and phloem are directed toward the inside and the outside of the stem respectively. Transverse and longitudinal views (fig. 12) throw additional light on the organization of these cortical bundles.

Course of leaf trace in adult stem.—Due to the difficulties involved in following up strands of such size, it is impossible to determine with certainty whether the arrangement of the leaf traces in the adult stem of *D. spinulosum* is the same as that found characteristic of the seedlings. The problem becomes increasingly difficult as the plant reaches an age when the crown comprises numerous developing leaves. From longitudinal and transverse sections of the adult stem, however, it is evident that the same general relation between lateral oblique traces and a horizontal girdling strand is maintained, but it is probable that the girdling is only partial, that traces a and a^1 (fig. 9) would have their origin at points more remote from each other in the adult stem. It is also probable that there is no appreciable increase in the number of traces associated with successive leaves, beyond the number described as supplying the leaves of the ten-year-old seedling (fig. 10).

Discussion

In his description of the girdling habit, METTENIUS (10) finds that "in the developmental stage the traces of the youngest leaves

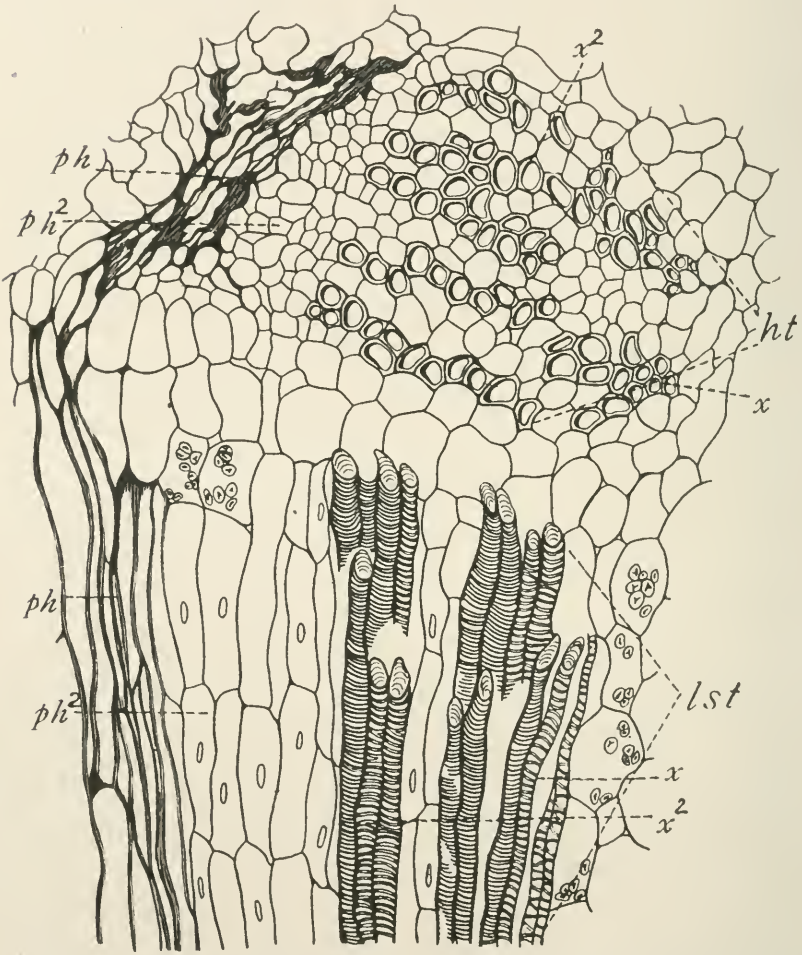

FIG. 12.—Detail of organization of cortical foliar strands of three-year-old plant: *ht*, horizontal strand in transverse section; *lst*, longitudinal section of vertical lateral trace near point of union with horizontal strand; *x*, primary sylem; x^2, secondary xylem; *ph*, primary phloem; ph^2, secondary phloem; ×120.

lie in the region of the vegetative point, and at first ascend in an almost perpendicular direction, but during further growth assume

gradually a horizontal position, and with subsequent growth are lengthened and the expanse increased." MATTE (9) and Sister HELEN ANGELA (3), in connection with *Ceratozamia*, both describe a similar vertical position of traces in the early developmental stages, girdling becoming evident with the increase in diameter of the inclosed group of leaves and stem. In more recent investigations of *Dioon edule* by THIESSEN, and of *D. spinulosum* by Sister HELEN ANGELA (4), however, both authors maintain that the girdles are established very early, and that their horizontal course is laid down from the beginning.

The results of the present investigation indicate two possibilities therefore. Either the arrangement of cortical strands in the older seedlings and adult wood of *Dioon spinulosum* differs from that found in the embryo and very young seedlings of both species of *Dioon*, or the preceding statements need considerable modification.

At the very tips of the two-, three-, and ten-year-old seedlings (fig. 10) the perpendicular arrangement of the lateral strands and their connection with the horizontal girdles is unmistakable. It is reasonable to suppose, therefore, that the arrangement of foliar strands in the first leaves of the young seedling would be substantially the same as that characterizing the leaves of the older seedlings, save that (1) the very young strands having their origin in the cotyledonary plate would ascend vertically for a shorter distance before anastomosing to form the horizontal girdles, and (2) there would be likely to be a decrease in the number of strands leaving the vascular plate for each leaf base.

Another question of importance is the significant relationship suggested by the distribution of leaf traces in the seedlings of *D. spinulosum*. Thus we find numerous strands (varying from 7 to 9) passing obliquely upward into each leaf base, each one of which causes a gap of its own in the main stele. As previously indicated, however, these strands do not all enter the petiole. There is instead an anastomosis of traces in the sheathing base of the leaf, resulting in the conspicuous and characteristic horizontal girdles, which correspond in many respects to the marginal vein of

the sheathing monocotyl leaves, save that the marginal vein of the typically sheathing monocotyl leaf is connected with a large number of bundles which come off around the entire periphery of the stem.

Summary

1. The medullary rays of *Dioon spinulosum* are of three distinct types: uniseriate rays, a single layer of cells wide and several cells deep; multiseriate rays, two to several cells in width and of variable longitudinal extent; and broad foliar rays or leaf gaps, which, with their included leaf traces, are such a constant feature of this wood.

2. The fibrovascular elements constituting the leaf traces in the foliar rays and connecting these traces with the secondary wood are peculiar, irregular scalariform tracheids which in the course of their development curve gradually downward through the ray, until they become inserted between the perpendicular fibrous elements of the main stele.

3. The regular pitted elements of the secondary xylem are also often diverted to one side into a direction parallel to that of the trace.

4. Both the scalariform and the pitted elements constituting these traces, in their peculiar manner of enlargement and elongation, furnish excellent illustrations of gliding growth.

5. For each leaf or scale leaf 7–9 strands (the number varying with the size of the leaf base) separate from the vascular cylinder. The two inner ones, arising from the proximal side of the central cylinder, pursue a more or less direct vertical course into the ventral part of the petiole; the rest of the traces, leaving the stem cylinder at different points, pass obliquely upward into the cortex and the sheathing base of the leaf, where an anastomosis of traces takes place, resulting in the two characteristic girdles.

6. The two direct strands entering the ventral or abaxial part of the leaf may also unite with the two dorsal girdling strands at the base of the petiole, so that the whole system is reducible in the older seedlings and adult stem to two main horizontal strands with their associated lateral traces.

Grateful acknowledgment is made of the helpful criticism and advice given by Professors JOHN M. COULTER, CHARLES J. CHAMBERLAIN, and W. J. G. LAND during the progress of the investigation. The writer is also greatly indebted to Dr. CHAMBERLAIN for the very generous supply of material.

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EXPLANATION OF PLATES XV–XVII

All the drawings were made with the aid of a camera lucida, except figs. 8, 9, 10, 11, which are diagrams showing origin and distribution of foliar vascular strands, as traced from cleared material, supplemented, where detail in connection was required, by serial sections; figs. 1–3 and 12 are in the text.

PLATE XV

FIG. 4.—Mature wood: radial longitudinal section of large foliar ray, showing organization of leaf trace and structure of scalariform tracheids of trace: *b*, bulbous bases of tracheids; *c*, calcium oxalate crystals; ×90.

FIG. 5.—Mature wood: radial longitudinal section near lateral border of leaf gap or foliar ray; *p*, pitted tracheids of secondary wood diverted to one side into direction parallel to that of trace; *sc*, scalariform tracheids of trace extending down between fibrous elements of main stele; ×55.

PLATE XVI

FIG. 6.—Mature wood: tangential section of foliar ray; *ll*, leaf trace, scalariform tracheids of trace seen in both longitudinal and transverse section; *x*, primary wood; *ph*, disorganized phloem; *mc*, mucilage duct; ×56.

FIG. 7.—Adult wood: tangential view of foliar ray in vicinity of cambium, tracheids constituting trace seen only in transverse section; ×56.

PLATE XVII

FIG. 8.—Two-year-old seedling, diagram showing: *sl*, origin and course of vascular supply of one of first scale leaves; *L*, first year foliage leaf, and l^2 second year foliage leaf, as traced from cleared material; *a*, a^1, *b*, b^1, *c*, c^1, etc., traces of scale leaf; *cs*, cotyledonary strands; *R*, vascular system of root; ×4.

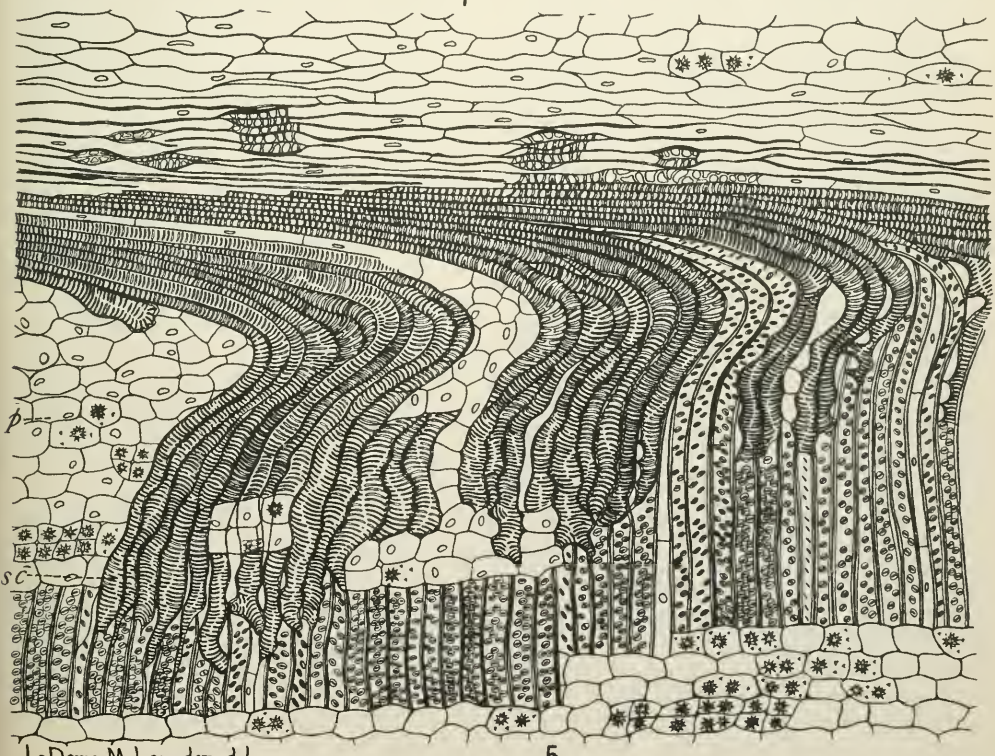
FIG. 9.—Three-year-old seedling: diagram showing connection of lateral traces, *a*, a^1, *b*, b^1, *c*, c^1, *d*, d^1, and *e*, with stem cylinder, and manner of anastomosis to form horizontal girdles; *A*, *D*, two of the four main cotyledonary bundles; *S*, *B*, two of the four principal groups of stem bundles; *lg*, leaf gaps corresponding to foliar gaps of adult stele illustrated in figs. 6, 7; ×4.5.

FIG. 9*a*.—Three-year-old seedling; ×0.5.

FIG. 10.—Median longitudinal section through apical portion of ten-year-old plant, showing only one side of the three sets of horizontal girdles with their associated lateral vertical strands; L_1, L_2, L_3, first, second, and third leaves of crown; second leaf not shown and only part of third leaf; l_1^1, l_1^4, horizontal strands entering adaxial part of leaf one; l_1^2, l_1^3, ventral strands apparently united with the two dorsal girdling strands l_1^1 and l_1^4; ×2.5.

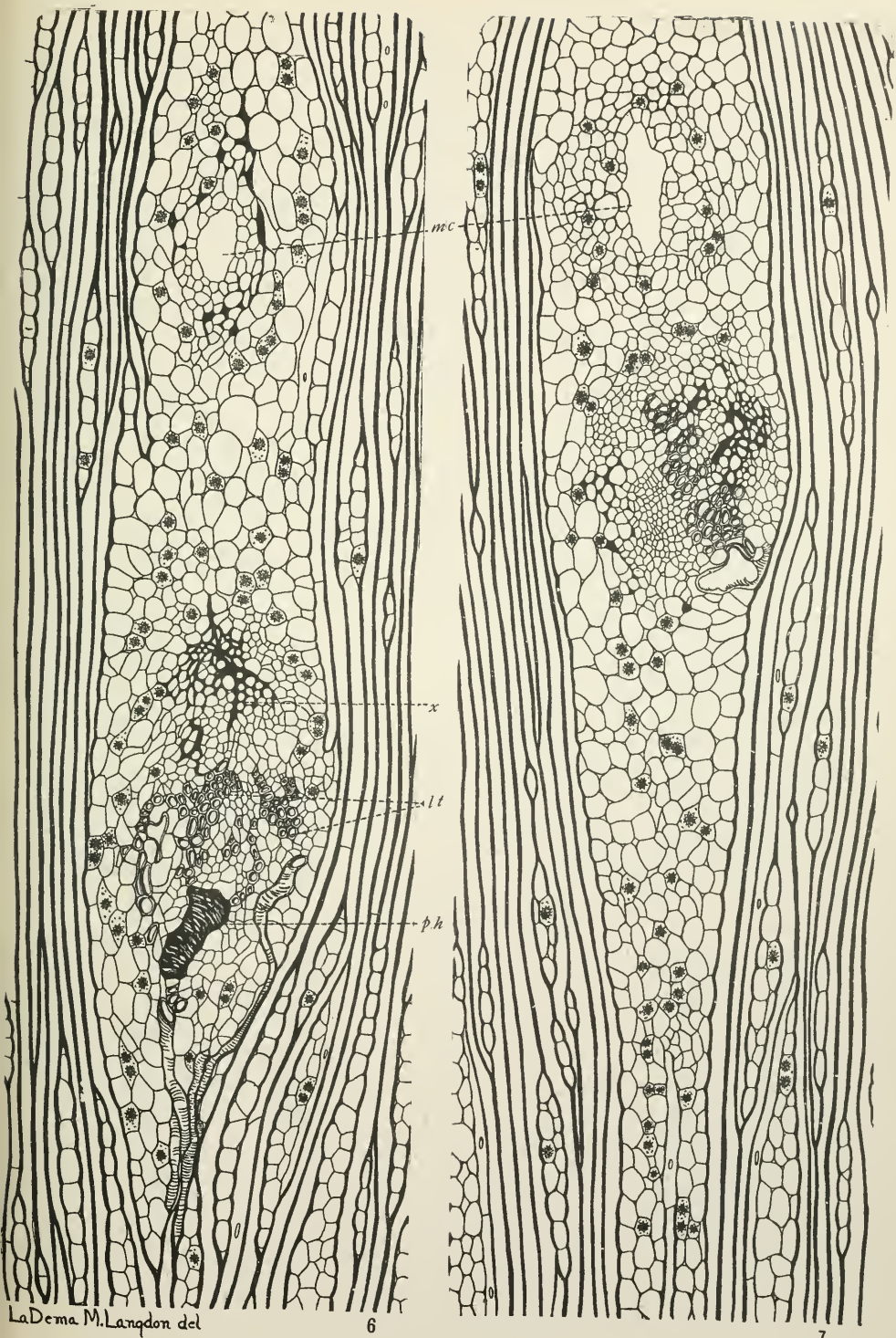
FIG. 11.—Diagram of entire vascular supply of first leaf, L_1, fig. 10; complete anastomosis of leaf traces to form one main horizontal girdling strand; ×2.5.

4



5

LANGDON on DIOON



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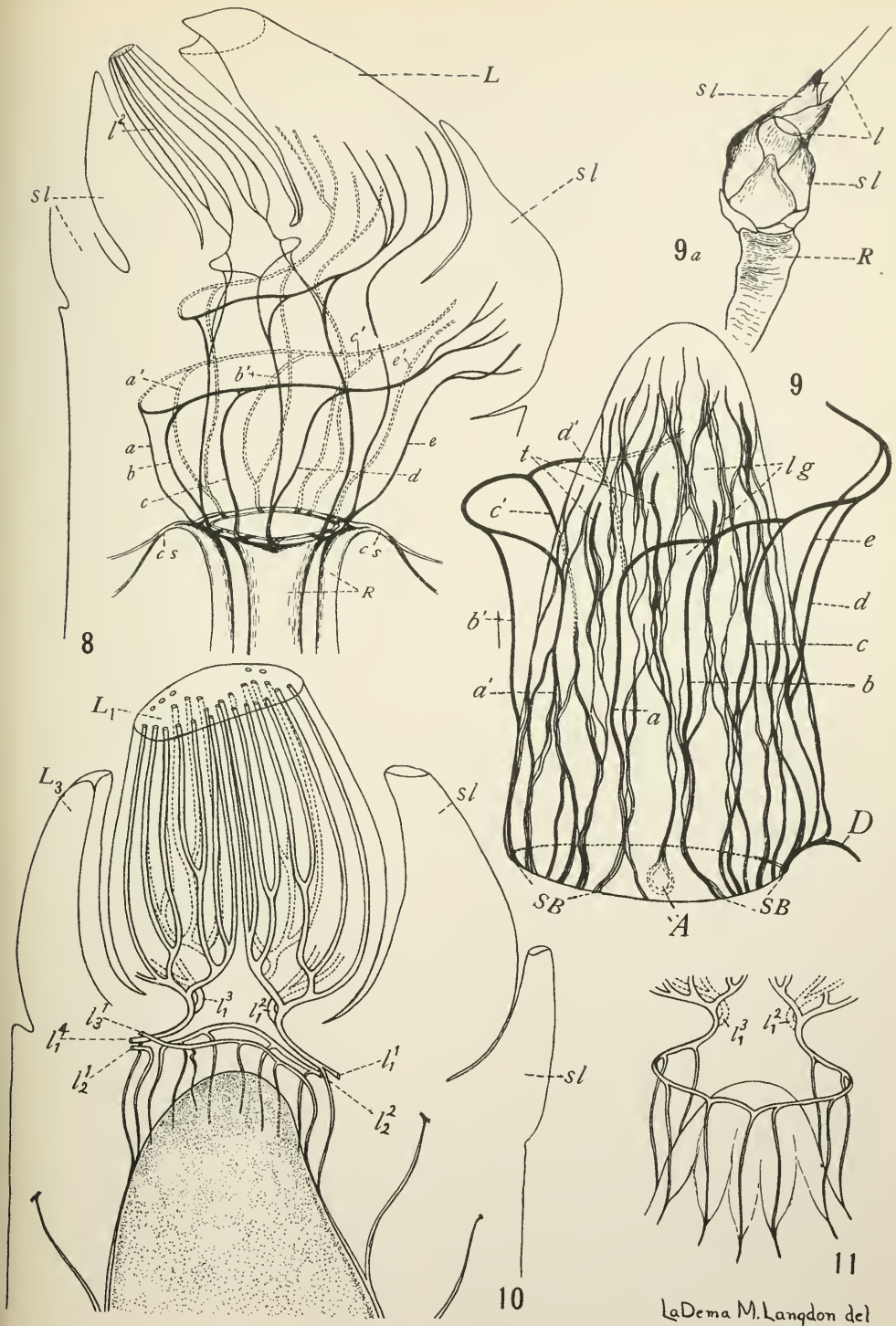

LANGDON on DIOON

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COMPOSITION OF GASES IN INTERCELLULAR SPACES OF APPLES AND POTATOES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 271

J. R. Magness

(WITH ONE FIGURE)

Introduction

During a study of the ripening processes in fruits, and the chemical and physiological changes associated with them, the question has arisen as to what may be the composition of the gas in the intercellular spaces. The gas within the tissues constitutes in part the medium in which the processes associated with the life of an organism take place. It is only reasonable to suppose that the composition of this medium may exert some influence upon the rate or nature of the changes taking place. The difficulty of extracting the gases from the interior of the tissues is probably responsible for the fact that plant physiologists have almost entirely neglected studies along this line. Gerber (5) reports work of Fremy published in 1840 and 1860, in which the gas contained in apples was analyzed at intervals during their development and ripening. He found oxygen more abundant in the green fruit, the amount decreasing as the fruit matured on the tree. We have, however, no critical studies upon the internal gases of plant tissues.

An apparatus has been devised for obtaining a sample of the gas from within the tissues, without contamination with air. It is the purpose of this preliminary report to describe the apparatus and methods of sampling, together with the data secured, in order that they may be available to workers along related lines.

Apparatus

The apparatus used in extracting the gas is shown in fig. 1. It consists of a leveling bottle or burette (*A*), connected through heavy walled rubber tubing to a side neck at the base of a thick-walled glass cylinder (*B*). This cylinder is flared at the top, and

fitted with a ground glass stopper (*C*) in which is sealed a capillary tube. The flare in the top of the cylinder should be sufficient to allow the stopper to set well down (as illustrated), in order to permit a mercury seal above the stopper. The capillary tubing is bent around in the manner shown, in such a way that the tip can be immersed in a vessel of mercury (*D*). Glass stopcocks (*E* and *F*) must be in the positions shown. It is especially important that the cock *F* be as indicated, rather than directly above the cylinder, for by the former arrangement any small leak about the cock can quickly be detected. Cocks and stopper should be kept well

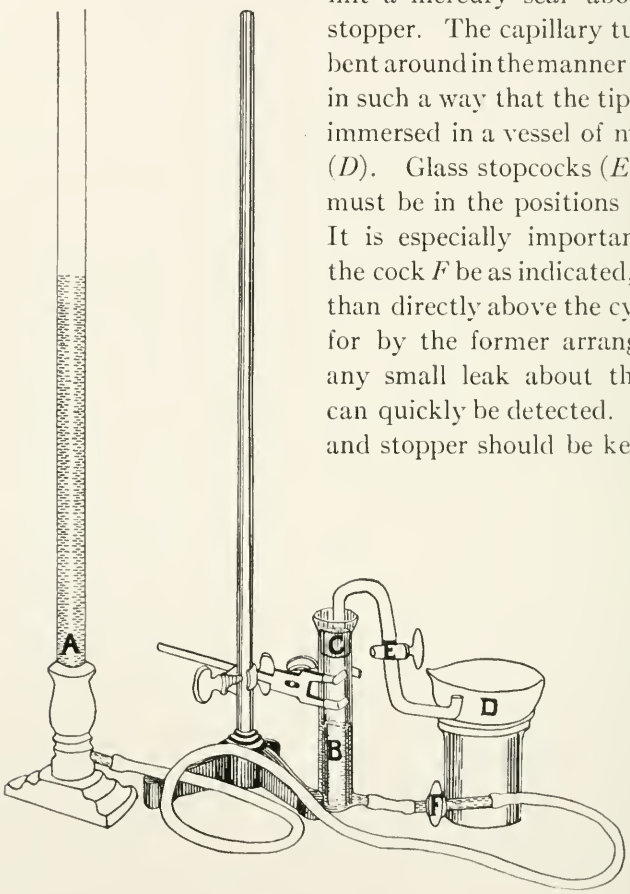

FIG. 1

coated with heavy desiccator grease. The proper dimensions for the cylinder (*B*) will obviously vary with the type of tissue being examined, and the volume of this tissue necessary to secure a gas sample adequate for an analysis. For work with apple and potato tissue, a cylinder 6 inches long and 1 inch inside diameter has been found very satisfactory.

Method

By opening the stopcock *E* and *F*, mercury was allowed to flow from the leveling burette into the cylinder until the latter was about two-thirds full. A plug of the tissue from which the gas was to be extracted was then cut with a cork borer. By using a sharp borer and cutting clean, the epidermal layers sealed each end of the plug of tissue, while all cut surfaces were in close contact with the walls of the cork borer. Consequently, there was no opportunity for contamination of the sample with the air.

The tip of the cork borer was then put under the mercury in the cylinder, and the sample plug of tissue pushed out under the mercury; a long glass rod has been found satisfactory for this. The plug was held beneath the mercury while the rod was replaced by a wire spring, and the ground glass stopper fitted into the cylinder. A mercury seal above the stopper precluded the possibility of leaks. The stopcocks (*E* and *F*) were again opened, and all air in the cylinder and capillary tubing replaced by mercury. The tip of the capillary was immersed under mercury in *D*, the cock (*E*) closed, and the leveling burette (*A*) lowered. The partial or almost complete vacuum in the cylinder causes the gas in the tissue to expand; it escapes from the tissue and collects in the top of the cylinder. The first few bubbles of gas were always discarded by driving them out through the capillary. When sufficient gas for the final analysis had collected in the top of the cylinder, it was driven off and collected over mercury in a small vial. The sample was transferred to a Bonnier-Mangin gas analysis apparatus of the type described originally by AUBERT (2) and later by GRAFE (4). CO_2 absorption was by means of 15 per cent KOH; oxygen was absorbed by 8 per cent pyrogallic acid in 30 per cent KOH. About one-half cc. of gas is sufficient for an analysis in this apparatus.

Results

Three boxes of Yellow Newton apples, representing three different trees at Watsonville, California, were used in the analyses of gas in apples. Some apples from each box were stored in a refrigerator at 6° C. and at 11° C. Others were stored in a vessel immersed in a water bath held at 20° C.; while a fourth lot was

held in an oven at 30° C. A few were held also at 2° C. In all cases abundant aeration was provided to prevent the possibility of an accumulation of CO_2 in the air surrounding the fruit. A summary of the data on the internal atmospheres in apples is given in table I.

TABLE I

ANALYSES OF GAS IN INTERCELLULAR SPACES OF YELLOW NEWTON APPLES

Temperature of storage °C.	Number of determinations	Percentage CO_2	Percentage O_2	Percentage CO_2+O_2	Percentage N_2 by difference
2	5	6.7	14.2	20.9	79.1
6	30	8.4	12.9	21.3	78.7
11	27	12.2	10.7	22.9	77.1
20	31	17.2	5.5	22.7	77.3
30	29	21.4	3.2	24.6	75.4

The data presented in table I require but little discussion. It is apparent that the percentage of CO_2 in the gas within the tissues increases markedly at the higher temperatures. At the same time there is a corresponding decrease in the percentage of oxygen present, the average ranging from 14.2 per cent at 2° C. to only 3.2 per cent at 30° C. These data, representing averages of a number of determinations, clearly indicate the marked variation that may occur in the composition of gas in the tissues under varying conditions of temperature.

It is of interest to note that at the lower temperatures the total percentage of oxygen plus that of carbon dioxide is about equal to that of the air. At the higher temperatures, however, and in association with the decreasing amounts of oxygen in the tissues, the sum of these two gases gradually increases. This would indicate that at the higher temperatures one molecule of oxygen liberates more than one molecule of CO_2. Such data accord with the work of GERBER (5), who found that in fleshy fruits stored at high temperatures acids were mainly respired, and that the ratio of CO_2 to O_2 under these conditions was considerably superior to unity. There is also the possibility that at the higher temperatures a certain amount of anaerobic respiration is going on, due to the relatively small amount of oxygen present. This would result in

an increased amount of CO_2 in the tissues, without a corresponding decrease in oxygen.

Table II gives the data obtained for potatoes. The potatoes used were purchased on the open market, and the variety was not determined. They were sound, smooth, and of uniform average size. A few carrots were also studied, for comparison with the apples and potatoes. From the data presented it is apparent that the same general tendency holds in potatoes and carrots that was noted in apples, that is, an increasing percentage of CO_2 and a decreasing oxygen content at higher temperatures.

TABLE II

ANALYSES OF GAS IN INTERCELLULAR SPACES OF POTATOES AND CARROTS

Temperature of storage °C.	Number of determinations	Percentage CO_2	Percentage O_2	Percentage CO_2+O_2	Percentage N_2 by difference
			Potatoes		
11	8	19.6	10.9	30.5	69.5
22	8	34.4	5.7	40.1	59.9
			Carrots		
11	2	12.2	13.1	25.3	76.7
24	2	28.6	5.2	33.8	66.2

It will be noted that the total CO_2 and oxygen is much higher in the case of potatoes than was found in apples. This variation may be due in part to the fact that there is relatively much less intercellular space in potatoes than in apples, and a higher percentage of the gas may have come out of solution in the juice in the samples obtained from potatoes than in those obtained from apples. It is necessary to use much larger samples of potato tissue in order to obtain sufficient gas for an analysis than is essential when apple tissue is used.

The amount of gas that may be coming out of solution in the juice, rather than from the intercellular spaces, presents a difficulty inherent in this method of sampling. There is no assurance that the gas that comes out of solution is of exactly the same composition as that of the intercellular spaces. The consistent results recorded

for fruit under the different temperatures tested, however, clearly indicate the tendency of the oxygen-carbon dioxide ratio within the tissues, regardless of the fact that the absolute values may vary somewhat.

Effect of wounding

Many references to the effect of wounding plant tissues upon rate of respiration are found in the literature. Invariably wounding of the tissue has resulted in an increased rate of respiration. GERBER has found this to be true of apples, grapes, and other fruits. APPLEMAN (1) has reported the same phenomenon for white potatoes.

A few apples were prepared for a study of the effect of wounding upon the composition of the internal atmosphere. A thin slice of the peel was removed from each end of the fruits, and they were then put in storage at the various temperatures by the side of whole fruits serving as checks. The data from the analyses of these fruits are reported in table III. It is apparent from these data that removing the epidermis greatly facilitates the entrance of oxygen to the tissues, and also the escape of accumulated CO_2. It would be interesting to know to what extent increased respiration following wounding is due to mechanically facilitating this gaseous exchange, and to what extent it is due to actual metabolic changes in the wounded tissues.

TABLE III

EFFECT OF REMOVING PEEL FROM ENDS OF FRUITS UPON COMPOSITION OF INTERNAL ATMOSPHERE

Temperature °C.	Treatment	Number of Determinations	Percentage CO_2	Percentage O_2	Percentage CO_2 + O_2
1.....	Whole apples	3	6.6	14.6	21.2
1.....	Ends peeled	4	1.7	15.8	17.5
20.....	Whole apples	2	17.8	7.0	24.8
20.....	Ends peeled	2	7.4	9.9	17.3
30.....	Whole apples	2	23.9	1.8	25.7
30.....	Ends peeled	2	12.6	8.9	21.5

Variation in composition of gases

Considerable variation occurred between individual apples or potatoes held under identical conditions. This is to be expected

when the wide variation in size, thickness of epidermis, etc., is considered. The extremes of variation found in apples held at 20° C. are indicative of the range of fluctuation that may be encountered in work of this type. The 31 apples analyzed at this temperature contained gas averaging 17.2 per cent CO_2. The minimum CO_2 recorded for any apple of the lot was 12.5 per cent; the maximum 25.7 per cent. Only one apple, however, showed more than 21.8 per cent, so that this latter figure is a more accurate maximum. The extremes of oxygen variation were somewhat less. With an average of 5.5 per cent oxygen, the minimum value was 1.0 per cent, and the maximum 9.5 per cent. Although most of the values were very much nearer the mean than these, it is essential that a considerable number of individual analyses be made to determine the true mean for any given condition.

Factors influencing amount

Three main factors operate to determine the amounts of CO_2 and oxygen in the intercellular spaces at any given temperature. These are (1) the rate of oxidation, or the rate at which oxygen is taken up from and CO_2 given off into the intercellular spaces; (2) the permeability of the skin or epidermal covering to CO_2 and oxygen; and (3) the difference in pressure of CO_2 and oxygen within and without the fruit, which determines the rate of gaseous exchange when the permeability factor is constant. The effect on each of these factors of varying the temperature will explain the variation occurring in the internal atmosphere of the tissues studied at the different temperatures.

Effect of temperature on oxidation processes.—Gore (6) has found that the rate of respiration for a large number of fruits, as measured by the quantity of CO_2 given off, increased, on an average, 2.38 times for a 10° rise in temperature. Enzymatic processes in general, within the range of temperatures here studied, show an increase of from two to three times for each 10° rise. It is thus apparent that the oxidative processes will be speeded up very markedly by temperature increases.

Effect of temperature on permeability.—Denny (3), in a study of the permeability of a number of plant membranes, has

found that in general the increase in permeability per 10° rise in temperature varies from 1.3 to 1.8 times, averaging about 1.5. These data are based on permeability to water, but there is no reason for believing that gases would be fundamentally different. The diffusion of gases in all probability is mainly a physical process, and as such is relatively much less affected by temperature changes than the chemical changes involved in oxidation.

From a consideration of these relative effects of temperature on oxidation and on permeability, it is apparent that the absorption of oxygen and release of CO_2 are increased much more by a given rise in temperature than is the tendency for oxygen to be supplied to the tissues, and CO_2 to be given off from them. Consequently, as the temperature is raised, the amount of oxygen in the tissues becomes less and less, while the CO_2 accumulates correspondingly. This continues until the third factor becomes effective, that is, the difference of CO_2 and oxygen pressures within and without the fruit becomes so great that equilibrium is again established.

Significance of ratio

No attempt has been made in this preliminary work to associate the percentages of CO_2 and oxygen found with the processes taking place in the fruit. The data presented, however, clearly indicate the necessity of taking this factor into consideration in many types of horticultural and physiological investigations. It should be given attention in studies of the effect of temperature upon the processes in plant tissues, for it is readily apparent that much variation may be caused by the composition of the medium in which these processes are carried on. Of special importance is the application of studies of this type to the questions as to the effect of wounding and various other treatments on the respiratory processes in tissues. Finally, it is of prime importance to know the composition of the internal atmosphere in studying the effects of various gases, etc., on plant organs. Some work has been done on the effect of various gases on fruits and vegetables in storage. Obviously it is essential in such work that the composition of the internal atmosphere be known.

The writer feels deeply indebted to Dr. WILLIAM CROCKER, and to Mr. W. S. BALLARD, U.S. Department of Agriculture, for valuable suggestions in regard to the apparatus for extracting the gas, and for many helpful suggestions during the progress of this work.

U.S. DEPARTMENT OF AGRICULTURE
WATSONVILLE, CAL.

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NOVEMBER 1920

NORTH AMERICAN SPECIES OF TARAXACUM[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 272

EARL EDWARD SHERFF

(WITH PLATES XXXI–XXXIII)

For many years our knowledge of the American species of *Taraxacum* has been in a very imperfect and chaotic state. The perusal of the more prominent manuals and floras issued in the United States during the past few decades shows a surprising confusion of forms and multiplicity of specific names. This confusion is easily accounted for by the fact that most of the *Taraxacum* forms tend strongly to intergrade, so much so that many botanists in the past have despaired of their specific segregation. Thus TORREY and GRAY (Fl. N. Amer. 2:494. 1843), after describing *Taraxacum dens-leonis* (=*T. vulgare*), wrote as an introduction to their four additional species: "The following species (the characters of which we copy from chiefly DE CANDOLLE, who keeps them distinct), as well as nearly all the genuine *Taraxaca*, are not improbably

[1] Including the West Indies, but not Greenland. The large number of new species recently proposed for Greenland by DAHLSTEDT have made it inadvisable to include the Greenland plants until a more abundant supply of Greenland material can be obtained for detailed study. So far, however, I have examined no plants from Greenland that were not clearly referable either to those species included in this treatment or to *Taraxacum nivale* Lange, a species close to *T. lyratum* but differing in having the achenes glabrous or nearly so. From a study of DAHLSTEDT'S work (Archiv f. Botanik 4^8:1–41. 1905; *ibid.*, 5^9:1–44. 1906) and those of his determinations accessible to me, it appears that his "new species" are mostly synonymous with *Taraxacum lyratum*, *T. nivale*, and *T. ceratophorum*.

correctly viewed by FRIES, KOCH, and other excellent botanists, as mere varieties of this, the *Common Dandelion*."[2] At a later date we find that GRAY himself (Synopt. Fl. N. Amer. 1²:440. 1884) had come to regard all the continental North American forms as representing varieties of but one species, which he stated to be a "very polymorphous species."

In 1907 there appeared the classic monograph of *Taraxacum* by HANDEL-MAZZETTI. This author, evidently impressed with the desultory treatment which the genus usually had been accorded in previous studies, quoted the relevant words of REICHENBACH (Fl. Germ. Excurs. 270. 1830–1832), which are here translated: "A genus seriously forsaken heretofore, because of the negligence of writers. A positively tedious comparison of leaves, without the remaining points having been carefully investigated and clearly set forth, renders the amateurs fully as confused as are the botanists themselves. Moreover, the fruits especially must be observed, and these only in their mature state."

In HANDEL-MAZZETTI's work there was given an admirable presentation of the various species of the genus. Even this valuable monograph, however, was rather inadequate for a critical opinion of the North American species, since there were a number of the more recently proposed species of which he obviously had not seen authentic specimens. In certain other cases his examination of American specimens was too limited, and I fail to find even the slightest mention of some of the species proposed by American authors previous to 1907. What seems worst of all, however, is that the many valid results of his research have been passed by almost unnoticed until the present day in this country. Taxonomic literature relating to *Taraxacum* in America is still weighted with inaccuracies that ought to be corrected.

The study presented herewith was undertaken in 1918. From the beginning the main purpose has been to correlate much of the material in American herbaria with HANDEL-MAZZETTI's treatment, to corroborate his results where possible, to correct or improve and to augment where there was need, and then to present

[2] For reference to the works of FRIES, KOCH, and VOITH, touching this point, see DC. Prodr. 7:145 (footnote). 1838.

the conclusions for their easier accessibility to American students. The full generic description given by HANDEL-MAZZETTI is omitted, nor has it been attempted to repeat his extensive lists of synonyms in full. Only such synonyms are given as seem vital or as were misplaced or overlooked by him. For the sake of comparisons, however, his excellent specific descriptions have been followed closely; in the main, only such alterations have been made as considerations of brevity or accuracy would dictate.

Since the completion of my research there has appeared the recent article by STORK (Bull. Torr. Bot. Club **47**: 199–210. *pls. 6, 7.* 1920). It contains interesting data concerning sexuality, variation, and cytological aspects of certain American species, and cites several references that must be omitted here.

As may be noted in the following pages, I have found no occasion for proposing a single new species. In fact the literature of the genus has suffered seriously in the past from the persistent and repeated proposal of ill-advised and scantily considered specific names, many of them founded upon freakish or even immature material. Rather have I been compelled to reduce three species retained by HANDEL-MAZZETTI (*T. phymatocarpum*, *T. mexicanum*, and *T. lapponicum*) to synonomy, thus giving a total of only five species in our range.

Most of the work was prosecuted in the Herbarium of Field Museum, where I was afforded the fullest measure of freedom and courtesy through the kindness of the Curator, Dr. CHARLES F. MILLSPAUGH. The entire collections of *Taraxacum* in the United States National Herbarium, especially rich in specimens from Alaska and the Arctic regions, were loaned through the generosity of the Associate Curator, Mr. WILLIAM R. MAXON. All of the highly important materials in the Herbarium of the Canadian Geological Survey were loaned by Professor JAMES M. MACOUN, late of that Herbarium. Among these were a vast number of valuable specimens from western Canada and the Arctic regions. All of the nearly 800 specimens in the Herbarium of Boissier and the Herbarium of the Institut de Botanique, Geneva, were loaned by Dr. R. CHODAT. These, while mostly from Europe, Asia, and elsewhere than from North America, were of the greatest value in

promoting a better evaluation of the North American species. On two occasions I was permitted by Dr. JULIUS NIEUWLAND to study freely the specimens, several of them types, in the Greene Herbarium at the University of Notre Dame. To all of these botanists I here express my great indebtedness and gratitude. Others to whom also I am grateful for assistance rendered are: Dr. N. L. BRITTON, Director of the New York Botanical Garden; Dr. J. M. GREENMAN, Curator of the Herbarium, Missouri Botanical Garden; Dr. AVEN NELSON, President, University of Wyoming; Professor W. W. ROWLEE, Cornell University; Professor D. B. SWINGLE, Botanist and Bacteriologist, Montana Agricultural Experiment Station.

Abbreviations used for Herbaria: Hb. Boiss., Herb. Boissier; Hb. Can., Herb. Canadian Geological Survey; Hb. Chi., Herb. University of Chicago; Hb. Field, Herb. Field Museum of Natural History; Hb. N.Y., Herb. New York Botanical Garden; Hb. U.S., United States National Hebarium.

Clavis specierum

Achaenia matura rubescentia

Folia plerumque gracilia, profunde laciniato-pinnatifida; involucri foliola exteriora plerumque patentia vel etiam recurvata.....5. *T. laevigatum*

Folia crassiora, minus profunde pinnatifida vel etiam integra; involucri foliola exteriora plerumque adpressa.....3. *T. eriophorum*

Achaenia matura non (nisi interdum versus apicem in num. 1) rubescentia

Achaenia matura nigrescentia (vel interdum versus apicem rubescentia) 1. *T. lyratum*

Achaenia matura aliusmodi

Involucri foliola plerumque adpressa et corniculis instructa 2. *T. ceratophorum*

Involucri foliola ecorniculata vel raro corniculis minimis instructa, exteriora patentia vel reflexa.....4. *T. vulgare*

1. TARAXACUM LYRATUM (Led.) DC., Prodr. 7:148. 1838; *Leontodon lyratus* Ledebour, Icon. Pl. Fl. Ross. ill. 5:27. *pl. 497*. 1834; Fl. Altaica 4:152. 1833; *T. phymatocarpum* Vahl, Fl. Danica 13 (fasc. 39):6. *pl. 2298*. 1840; Watson, U.S. Geol. Explor. 40th Parallel 207. 1871; *T. laevigatum* Gray, Proc. Acad. Phil. 1863, p. 70 (non Willd.); *T. officinale* var. *scopulorum* Gray, Synopt. Fl. N. Amer. 1²:440. 1884; *T. Taraxacum* var. *scopulorum* Heller,

Cat. N. Amer. *pl. 8.* 1898; *T. scopulorum* Rydb., Mem. N.Y. Bot. Gard. 1:455. 1900; *T. rupestre* Greene, Pittonia 4:229. (Jan.) 1901; *T. alaskanum* Rydb., Bull. Torr. Bot. Club 28:512. (Sept.) 1901; *T. hyperboreum* Dahlst., Videsk.-Selsk. Christ. Math.-Naturv. Kl. 1909: no. 8. p. 26. 1910; *T. eurylepium* Dahlst., *loc. cit.* 72; *T. fasciculatum* Nels., Bot. Gaz. 56:71. 1913 (ex descript. et altitudine); *Leontodon rupestre* Rydb., Fl. Rocky Mts., 1035. 1917; *L. scopulorum* Rydb., *loc. cit.*—Pl. XXXI.

Herba valde variabilis, pusilla (interdum minima), 2–15 (–25) cm. alta. Radix tenuiuscula vel crassa, simplex vel multiceps, collo subsquamato vel foliorum vetustorum fragmentis persistentibus squamato, glabro vel sparse piloso vel sparse longo lanuginoso. Folia suberecta vel patentia, tenuia, glabra, lanceolata vel spathulata, 3–15 mm. lata, infra saepe longe attenuata, nunc integra, nunc denticulata vel sinuato-dentata, nunc regulariter incisa, lobis triangularibus vel lanceolatis, acutis vel obtusis, integris vel subdenticulatis, subrecurvis vel patentibus. Scapi plerumque pauci vel singuli, tenues, glabri vel iuveniles sub capitulo densiuscule villosi, florendi tempore foliis breviores vel multo longiores. Capitula minima (circum 7–12 mm. longa nec latiora) vel maiora (circum 12–18 mm. longa et paulo latiora). Involucri foliola pauca, atroviridiora vel nigricantia, ecorniculata vel corniculis parvis saepe instructa; exterioris seriei foliola adpressa vel laxe adcumbentia, interiorum angustiorum longitudinis 0.4–0.5 attingentia, infima vix breviora, omnia latius angustiusve ovata (latitudine 0.5–1.5 longiora), margine decolorato variabili vel nullo. Flores pauci vel numerosi, sulphurei vel flavi, involucro 1–4 mm. longiores. Achaenia maiuscula, ad 5 mm. longa, atra, fere nigra (versus apicem saepe subrubida), iuniora brunnea, tota rugosa, tuberculis largis, supra longioribus, crassis tenuioribusve obsita, abrupte in cuspidem crasse cylindricam, sulcatam, brevem vel brevissimam, totius fructus septimam partem aequantem vel quintam vix superantem contracta. Rostrum strictum, achaenio subaequilongum. Pappus illo paulo longior, albus.

Distribution.—Greenland, Arctic America, and northeastern Asia, southward at alpine heights along the mountains of western Canada and the western United States to Arizona.

SPECIMENS EXAMINED.—Alaska (and neighboring islands): Hall Island, July 14, 1899, *Brewer* and *Coe* 427 (Hb. Greene 30831); Point Gustavus, Glacier Bay, June 10–12, 1899, *Coville* and *Kearney* 732 (Hb. U.S. 376695); Haenke Island, Disenchantment Bay, June 22, 1899, *iidem* 1097 (Hb. U.S. 376702); St. Matthew Island, Bering Sea, July 15, 1899, *iidem* 2164 (Hb. U.S. 376718); Bennet, July 31, 1907, *Henry C. Cowles* 1005 (Hb. Field 419544); Clifton Point, Dolphin-Union Straits (lat. 69° 13′N.), in 1916, Rev. *H. Girling* (Hb. Can. 99071); Herald Isl., Arctic Ocean, in 1881, *Capt. C. L. Hooper* (Hb. U.S. 424060); Wollaston Land (lat. 69–70° N., long. 115° W.), July 1915, *D. Jenness* 415 (Hb. Can. 98711); Camden Bay (lat. 70° N., long. 145° W.), Collinson Point, July 17, 1914, *Frits Johansen* 115 (Hb. Can. no 98716 in Hb. Field 483378); Bernard Harbor (lat. 68° 47′ N., long. 114° 46′ W.), August 1914, *idem* 276 (Hb. Can. 98715); Cape Bathurst (lat. 70° 35′ N., long. 128° 6, W.), July 1916, *idem* 508 (Hb. Can. 98713); Popof Isl., Shumagin Isls., July 14, 1899, *Trevor Kincaid* (Hb. U.S. 376796); Muir Glacier, July 8–19, 1899, *idem* (Hb. U.S. 376795, peculiar form with foliage of the *T. alaskanum* form and with achenes brown as in *T. ceratophorum*); Point Barrow (lat. 71° N.), steep side bank facing the ocean, July 23, 1898, *E. A. McIlhenny* (Hb. N.Y.; Hb. Can. 26283; type and cotype of *T. alaskanum* Rydb.); Point Barrow, in 1883, *Dr. John Murdoch* (Hb. U.S. 424062, 424063, and 424064, topotypes of *T. alaskanum* Rydb.); Kadiak Isl, vicinity of Karluk, July 5, 1903, *Cloudsley Rutter* 197 (Hb. U.S. 420615, the form matching LEDEBOUR's type illustration very closely); vicinity of Port Clarence, Teller Reindeer Station, tundra banks near beach July 20, 1901, *F. A. Walpole* 1492 (Hb. U.S. 378605), August 9, 1901, *idem* 1791 (Hb. U.S. 378905), and September 4, 1901, *idem* 1987 and 1988 (Hb. U.S. 379107 and 379108 respectively); vicinity of Port Clarence, banks along streams, flat west end of Tasuk Lagoon, August 22, 1901, *idem* 1895 (Hb. U.S. 379011); Arakamtchetchene Isl., Bering Staits, *C. Wright* in 1853–1856 (Hb. U.S. 424059).

British Columbia: West Summit of S. Kootenay Pass, mountain slopes, July 26, 1883, *Dawson* (Hb. Can. 15115); Chilliwhack River, alt. 6000 ft., rocky slopes, August 29, 1901, *J. M. Macoun* (Hb. Can. 26811); Hazelton, Skeena River, mountains, alt. 4500 ft., July 13, 1917, *idem* (Hb. Can. 98703; Hb. Field 483397); second summit west of Skagit River, July 24, 1905, *idem* (Hb. Can. 77001); Cascade Range, near head of McGillivray Creek, alt. 6500 ft., August 12, 1916, *idem* (Hb. Can. 98701; Hb. Field 483396); Mt. Queest, alt. 6000 ft., crevices of rocks, July 25, 1889, *idem* (Hb. Can. 15111; type of *T. rupestre* Greene); Kicking Horse Lake, alt. 8000 ft., alpine slopes, August 14, 1890, *idem* (Hb. Can. 11114; Hb. U.S. 219543); Kicking Horse Lake, damp open thickets, July 28, 1885, *John Macoun* (Hb. Can., 15110); Avalanche Mt., Selkirk Mts., alt. 8000 ft., August 4, 1890, *idem* (Hb. Can., *sine num.*); Revelstoke, alt. 1600 ft., July 26, 1905, *C. H. Shaw* 1008 (Hb. U.S. 621912); Tete Jaune Cache, headwaters of Fraser River, mountain summits, August 31, 1898, *W. Spreadborough* (Hb. Can. 19743).

Alberta: Below and at Ottertail Pass (Rocky Mt. Nat. Park), alt. 6900 ft., August 10, 1904, *John Macoun* (Hb. Can. 65620; Hb. Field 222849); Crow Nest Pass, mountain slopes, alt. 7000 ft., August 6, 1897, *idem* (Hb. Can. 23109); Fitzhugh Mt., Jasper Park, alpine summits, alt. 7000 ft., August 8, 1917, *J. M. Macoun* (Hb. Can. 98682, 98683 and 98684; Hb. Field 483384); Shovel Pass, Jasper Park, high slopes and summits, alt. 7000 ft., August 10, 1918, *idem* (Hb. Can. 98679; Hb. Field 483381); Shovel Pass, Jasper Park, among rocks at foot of cliff, alt. 6000 ft., August 17, 1918, *idem* (Hb. Can. 98680; Hb. Field 483382); Goat Mt., Jasper Park, above tree limit, alt. 7000 ft., July 18, 1918, *idem* (Hb. Can. 98681; Hb. Field 483383); Mt. Edith Cavell, Jasper Park, damp flat, alt. 6000 ft., *idem* (Hb. Can. 98690; Hb. Field 483389).

Montana: Old Hollowtop, near Pony, July 9, 1897, alt. 9000 ft., *Rydberg* and *Bessey* 5294 (Hb. U.S. 361402); above Stanton Lake, alt. 7000–7500 ft., August 1–7, 1894, *R. S. Williams* 1073 (Hb. Greene 48454; Hb. U.S. 288541).

Wyoming: Big Horn Mountains, alt. 10000 ft., July 17, 1890, anonymous (Hb. Greene 48456); without locality, *F. Tweedy* 745 pro parte (Hb. U.S. 41953).

Colorado: Mt. Hesperus, alt. 11000 ft., July 2, 1898, *Baker, Earle,* and *Tracy* 293 (Hb. Field 76097; Hb. Chi. 356356; Hb. U.S. 337212); Saguache (Sawatch) Range, alt. 12000 ft., August 1880, *T. S. Brandegee* (Hb. Field 204736); Uncompahgre River, mountain slopes, alt. 12000–13000 ft., August 1893, *C. A. Purpus* 719 (Hb. Chi. 357798).

Utah: La Sal Mts., alt. 3000–3300 m., July 7, 1911, *Rydberg* and *Garrett* 8720 (Hb. Can. 85360; Hb. U.S. 765075); Uintah Mts., above Bear River, alt. 12000 ft., August 1869, *Sereno Watson* 724 (Hb. U.S. 41943).

Nevada: Rocky Mountains, July 20, 1896, *Edward L. Greene* (Hb. Greene 48455).

Arizona: San Francisco Mts., August 27, 1889, *F. H. Knowlton* 142 (Hb. U.S. 41949).

Ledebour founded his species upon Asiatic material with immature fruit, collected in stony places upon an alpine summit along the Tschuja River opposite the mouth of the Tschegan River. Several of the specimens from Alaska (for example, *Coville* and *Kearney* 1097, *Rutter* 197) match his description and plate, also specimens of the type collection (legit Bunge, Hb. Boiss.) very closely. Many other Alaskan specimens fail to have the lateral laciniae of the leaves ovate, as described by Ledebour, but acute instead. Here must be placed *T. alaskanum* Rydb., of which I have examined the type sheet, also the cotype in the Herbarium of the Canadian Geological Survey. Proceeding south from Alaska, forms may be found coming from the high alpine

altitudes of Colorado, Utah, etc., that in some cases look even specifically distinct. Such plants (for example, *Baker*, *Earle*, and *Tracy* 293, *Tweedy* 745 pro parte) are commonly dwarfed, 2–3.5 cm. high, and their diminutive involucres measure sometimes as low as 4–6 mm. in width at base during anthesis. It is these plants that GRAY named *T. laevigatum*, and later *T. officinale* var. *scopulorum*. A study of numerous other specimens, however, especially from Montana, Alberta, and British Columbia, reveals all possible intergradations between the two extremes of foliage and involucre. One of these forms is the *T. rupestre* Greene, of which I have studied the type and all the other material cited by GREENE.

Recently RYDBERG (*loc. cit.*) has created the name *Leontodon scopulorum* for the dwarf alpine forms of the Rocky Mountains, but, as both HANDEL-MAZZETTI and I have finally concluded, this dwarf form is entirely inseparable from *T. lyratum*. Also, for those who discard the name *Taraxacum* but persist in employing the name *Leontodon*, the name *Leontodon lyratus*, as it was originally published by LEDEBOUR, should suffice.

HANDEL-MAZZETTI had seen no mature fruit of the materials regarded by him as *T. lyratum;* but a duplicate (*Jas. M. Macoun*, Mts. at Kicking Horse Lake, British Columbia, Hb. U.S. 219543) of one of the specimens cited by himself (and seen by him at the University of Vienna) has several mature achenes, which are black. Numerous other Canadian specimens examined have likewise black or blackish achenes, but in certain cases these achenes are slightly reddish near the top. At times sheets of material are observed on which the specimens have variously few, many, or all of their leaves spatulate or lanceolate, with margins merely dentate or even subentire. Typical examples of this kind are: *Knowlton* 142, Arizona (Hb. U.S. 41949); *Macoun*, British Columbia (Hb. Can. 98701; Hb. Field 483396); *Coville* and *Kearney* 1097, Alaska (Hb. U.S. 376702); *Walpole* 1791, 1895, and 1987, Alaska (Hb. U.S. 378905, 379011 and 379107 respectively). These specimens are extremely important, for some of them match the specimens of *T. phymatocarpum* from Greenland so minutely that all attempts at separation are fruitless. HANDEL-MAZZETTI (*loc. cit. pl.* 7) presents a distributional map in which he shows *T. lyratum* ran-

ging from southern Colorado northwestward through British Columbia, Alaska, and barely touching Asia.[3] For *T. phymatocarpum* he gives a more northern range, extending from Greenland westward through Alaska and slightly into Asia. My own study, however, leaves me entirely unable to maintain such a separation. To do so would necessitate in many instances actually taking materials on the same sheet, collected at the same time and place, and known to be even racially the same, and dividing them arbitrarily between the two "species," a manifestly absurd and indefensible procedure. In this connection it is interesting to note that, years ago, SERENO WATSON determined a specimen collected by himself in Utah (*Watson* 724, Hb. U.S. 41943) as *T. phymatocarpum* Vahl. He stated expressly on the label that his determination was "fide speciminis in Groen. a Rink lecti." Thus WATSON likewise was convinced of the identity of the Utah material with that of Greenland.[4]

T. fasciculatum Nels. was described from flowering specimens collected by *Alfred A. Griffin* (no. 111) from Wagon Wheel Gap, Blue Park, Colorado, alt. 11000 ft., July 21, 1912. NELSON has been unable to locate the type specimen for me, but the description ("few-several oblanceolate or oblong obtusish merely dentate or denticulate subsessile or short-petioled glabrous leaves 4–7 cm. long"), together with the high altitude recorded, indicates clearly that the plant was *T. lyratum* of the form that, from Greenland, has heretofore been termed *T. phymatocarpum*.

Occasionally a form of *T. lyratum* is found closely simulating the form of *T. ceratophorum* which GREENE described as *T. mutilum*, and differing clearly from "*T. mutilum*" only in having black achenes (for example, *Walpole* 1791 and 1987, Alaska, Hb. U.S. 378905 and 379107 respectively; *Dr. Murdoch*, Alaska, Hb. U.S. 424062 and 424064). Its foliage is long linear or linear-lanceolate, remotely and very sharply toothed. This form matches very closely the type illustrations of *T. hyperboreum* Dahlst., from Gjöa

[3] The type of *T. lyratum*, however, was collected in the interior of Asia!

[4] Elsewhere (U.S. Geol. Explor. Fortieth Parallel 207. 1871) WATSON said: "The present specimen, a single one only, is rather larger than those from Greenland, but is plainly the same plant."

Harbor, lat. 68° 37′ 38″ N., long. 96° 23′ 40″ W., and *T. eurylepium* Dahlst., from Herschell Island (cf. pl. XXXI fig. *c*). DAHLSTEDT had seen no achenes for either of his two proposed species, but a study of *Walpole* 1987 reveals the black achenes, as in typical *T. lyratum*. Numerous variations in foliage and involucre connect the form clearly with true *T. lyratum*, and make it impossible to draw any specific distinctions.

2. TARAXACUM CERATOPHORUM (Led.) DC. Prodr. **7**:146. 1838; *Leontodon ceratophorus* Ledebour, Icon. Pl. Fl. Ross. **1**:9. *pl. 34.* 1829; Fl. Altaica **4**:149. 1833; *T. montanum* Nutt. (non Mey. et DC.), Trans. Amer. Phil. Soc. n.s. **7**:430. 1841; WOOTON and STANDLEY, Contrib. U.S. Nat. Herb. **19**:627. 1915; *T. lividum* Heller, Bull. Torr. Bot. Club **24**:480. 1897 (exclud. synon. Waldst. et Kit.); *T. Chamissonis* Greene, Pittonia **4**:228. 1901; *T. lacerum* Greene, *loc. cit.* 230; *T. dumetorum* Greene, *loc. cit.* 230; *T. mutilum*, Greene, *loc. cit.* 239; *T. leiospermum* Rydb., Bull. Torr. Bot. Club **32**:137. 1905; *T. oblanceolatum* Nels. ex Rydb., Fl. Colorado 410. 1906 (ex synon. *T. dumetorum* Greene);[5] *T. lapponicum* Handel-Mazzetti, Monogr. Taraxacum 73. 1907 (saltem quantum ad plantas americanas, forsan non Kihlm.); *Leontodon dumetorum* Rydb., Fl. Rocky Mts. 1035. 1917; *L. leiospermum* Rydb., *loc. cit.*; *L. monticola* Rydb., *loc. cit.*—Pl. XXXII.

Herba valde polymorpha, plerumque robustior, 7–25 (–35) cm. alta. Radix crassiuscula, nigrescenti-corticata, collo haud vel vix squamato, glabro vel sparsissime lanato. Folia laxe procumbentia, adscendentia vel erecta, herbacea, viridia vel pallida, glabra vel infra sparsissime pilosa, lanceolata vel oblanceolata, 1–6 (–9) cm. lata, infra saepe longe attenuata, ad apicem acuta vel obtusa, leviter sinuato-dentata vel variis modis runcinato-incisa, raro integra vel tenuissime dissecta, lobis acutis, latius angustiusve triangularibus, integris vel dentatis, acutis, lobo terminali plerumque maiore. Scapi singuli vel numerosi, suberecti, florendi tempore foliis plus minusve aequilongi, denique elongati, iuveniles plus minusve lanato-pilosi. Capitula magna, 1.5–2.5 cm. alta et 2–5 cm. lata. Involucrum griseo-viride vel nigrescens, interdum pruinosum. Involucri foliola corniculis plus minusve

[5] AVEN NELSON 8236, distributed by NELSON as *T. oblanceolatum*, is likewise referable to *T. ceratophorum*.

atratis et apicem dilatatum saepe superantibus fere semper instructa, exteriora adpressa vel patentia, late ovata vel lanceolata, interiorum longitudinis $\frac{1}{6}$–$\frac{3}{5}$ (vel raro totum) aequantia, plerumque 5–15 mm. longa, margine decolorato interdum nullo sed saepius praesente et bene distincto. Flores numerosi, magni, foliolis 5–10 mm. longiores, flavi vel sulphurei. Achaenia 4–5 mm. longa, straminea vel brunnea vel griseo-brunnea, supra tuberculis angustis mediocris longitudinis dense obsita et saepe tota rugulosa, in cuspidem crassam vel angustam, brevem vel tertiae parti totius fructus aequantem cuneate attenuata. Rostrum tenue, achaenio paulo vel multo longius. Pappus albus, 5–8 mm. longus.

DISTRIBUTION.—Labrador and Alaska southward at higher altitudes to New Hampshire, Massachusetts, Montana, New Mexico, and California; in the entire Arctic region, the mountains of Central Asia, and even "in the Caucasus and in the Alps of Switzerland (a single locality)."

SPECIMENS EXAMINED.[6]—Labrador (Peninsula): Northern Labrador along the Ungava River, August 20, 1896, *Spreadborough* (Hb. Can. 14395); Ungava, *Lucien M. Turner* 613 (Hb. U.S. 222756).

Quebec: Banks of the Grand River, Gaspe County, June 30–July 3, 1904, *M. L. Fernald* (Hb. Field 465065; Hb. U.S. 605794); Rimouski County, July 4, 1907, *Fernald* and *Collins* 1210 (Hb. Can. 86493).

Keewatin: West Coast of Hudson Bay, lat. 56° N., sandy grounds, August, 1886, *James M. Macoun* (Hb. Can. 15112); Churchill, Hudson Bay, lat. 58° 50′ N., July 26, 1910, *idem* (Hb. Cornell Univ.; Hb. Can. 79286; Hb. Field 295238; important as matching exactly the form described by GREENE for his *T. mutilum*).

Manitoba: Birtle, vicinity of, along G.T. Pacif. R.R., June 26, 1906, *Macoun* and *Herriot* (Hb. Can. 77046); Forest, six miles east of, along G.T. Pacif. R.R., June 19, 1906, *iidem* (Hb. Can. 77047); Oak River, along G.T. Pacif. R.R., June 21, 1906, *iidem* (Hb. Can. 77048).

Mackenzie: Cape Barrow (south coast of Coronation Gulf), August 9, 1915, *Cox* and *O'Neil* 451 (Hb. Can. 98712; Hb. Field 483375); Fort Resolution, July 14, 1903, *Edward A. Preble* 210 (Hb. U.S. 421694).

[6] Many specimens are omitted for lack of space. As representing the extreme form with bracts ecorniculate (*T. lapponicum*), there may be added the following examples: Alberta: Near Old Man's River, damp grassy places, August 4, 1883, *Dawson* (Hb. Can. 15124). Wyoming: Northwestern part of state, August 9, 1893, *J. N. Rose* 679 (Hb. U.S. 41951). Utah: Tate Mine, near Marysvale, alt. 9000 ft., August 22, 1894, *Marcus E. Jones* 5853 (Hb. U.S. 233114); Gold Basin, La Sal Mountains, alt. 3000–3300 m., July 11, 1911, *Rydberg* and *Garrett* 8836 (Hb. U.S. 765101). California: Bear Valley, San Bernardino Mountains, in meadows, August, 1882, *S.B.* and *W. F. Parish* 1461 (Hb. Field 208755; Hb. U.S. 783095); Bear Valley, San Bernardino Mts., alt. 6500 ft., June 18, 1894, *S. B. Parish* 3131 (Hb. U.S. 21437).

Saskatchewan: Moose Jaw, open ground by the creek, June 20, 1896, *John Macoun* (Hb. Can. 12737); Moose Jaw, vicinity of, July 13, 1895, *idem* (Hb. Can. 11713); Prince Albert, camp thickets, June 29, 1896, *idem* (Hb. Can. 12283); Wood Mountain Post, thickets, June 11, 1895, *idem* (Hb. Can. 11712); Cypress Hills, thickets, June 24, 1894, *idem* (Hb. Can. 5087; labeled in GREENE's handwriting as being "part of type" of his *T. dumetorum*); Cypress Hills, springy places, June 2, 1884, *J. M. Macoun* (Hb. Can. 15131).

Assiniboia: Medicine Hat, June 8, 1894, *John Macoun* (Hb. U.S. 232067).

Montana: Bridger Mountains, alt. 7000 ft., June 14, 1897, *Rydberg* and *Bessey* 5295 (Hb. Can. 40007; Hb. Field 81947; a form having atypic foliage, possibly a hybrid); Midvale, plains, June 17, 1903, *L. M. Umbach* 75 (Hb. Field 191120; Hb. U.S. 541438); Highwood Mts., June 19, 1888, *R. S. Williams* 434 (Hb. U.S. 288542).

Wyoming: Yellowstone National Park, July 13, 1902, *Edgar A. Mearns* 1779 (Hb. U.S. 486830); Pacific Creek, 65 miles north of Point of Rocks, June 22, 1901, *Merrill* and *Wilcox* 575 (Hb. U.S. 580684).

Colorado: Ruxton Dell, alt. 2900 m., July 17, 1903, *F. E.* and *E. S. Clements* "363.1" (Hb. U.S. 580390); Camp Creek, Larimer County, semi-meadow land, July 6, 1903, *Leslie N. Goodding* 1462 (Hb. U.S. 581396); without locality (lat. 39–41° N.), in 1862 *Hall* and *Harbour* 357 (Hb. Field 17783 314685; Hb. U.S. 41940); Tennessee Pass, Lake County, July 10, 1902, *George E. Osterhout* 2645 (Hb. N.Y.; type of *Taraxacum leiospermum* Rydb.); Gray's Peak, vicinity of, alt. 12000 ft., August 1882 and 1885, *Patterson* and *Beaty* (Hb. Field 209706); Georgetown, vicinity of, June 28–August 7, 1875, *Harry N. Patterson* (Hb. Field 208950); Cuchara River, below Laveta, alt. 2100 m., May 28, 1900, *Rydberg* and *Vreeland* 5540 (Hb. Greene 48459); South Park, July 1873, *John Wolf* 268 (Hb. U.S. 41954); Central Colorado in 1873, *idem* 669 (Hb. Field 211601).

New Mexico: Santa Fe Canyon, 9 miles east of Santa Fe, alt. 8000 ft., June 2, 1897, *A. A.* and *E. Gertrude Heller* 3642 (Hb. Greene 48457; Hb. U.S. 306394; the basis, as to material examined and not as to synonomy Waldst. and Kit., of the name *Taraxacum lividum* Heller); Pecos River National Forest, at Winsor's Ranch, alt. 8400 ft., June 29, 1908, *Paul C. Standley* 4022 (Hb. U.S. 498416); Cloudcroft, June, 1912, *Elmer Stearns* 356 (Hb. U.S. 691021); Cloudcroft, vicinity of, June 30, 1899, *E. O. Wooton* (Hb. U.S. 739580 and 739583); Cox Canyon, Sacramento Mts., August 9, 1899, *idem* (Hb. U.S. 562510, 735339, and 739582); Silver Spring Canyon, Sacramento Mts., July 6, 1899, *idem* (Hb. U.S. 739581); Winter Folly, Sacramento Mts., August 13, 1899, *idem* (Hb. U.S. 735338).

Alaska (and neighboring islands): Fort St. Michaels, Norton Sound, June 23, 1865–1866, *H. M. Bannister* (Hb. Cornell Univ.; Hb. Field 301948); St. Paul Isl., July 9, 1899, *L. J. Cole* (Hb. U.S. 376691); Kadiak, July 2, 1899, *idem* (Hb. U.S. 376690); Kukak Bay, July 1–5, 1899, *Coville* and *Kearney* 1524 and 1690 (Hb. U.S. 376708 and 376711); Hall Island, July 14, 1899, *idem* 2028 (Hb. U.S. 376716); Unalaska, July 8, 1899, *iidem* 1721 (Hb. U.S.

376713); Attu Isl., June 26, 1873, *W. H. Dall* (Hb. U.S. 424065); Unalaska, July 11, 1892, *B. W. Everman* 69 (Hb. U.S. 376727); Dutch Harbor, Unalaska Isl., July 17, 1899, *B. E. Fernow* (Hb. Cornell Univ.); Johnson River, between Cook Inlet and the Tanana River, June 27, 1899, *E. F. Glenn* (Hb. U.S. 376755; type material of *Taraxacum mutilum* Greene); Iliamna River, Lake Iliamna region, open woods, June 29, 1902, *M. W. Gorman* 80 (Hb. U.S. 420101); Copper Center, vicinity of, in 1908, *C. W. H. Heideman* 78 (Hb. U.S. 421973); Unalaska, *A. Kellogg* 301 (Hb. U.S. 424067 and 424068); Popof Isl., Shumagin Isls., July 8–19, 1899, *Trevor Kincaid* (Hb. U.S. 376794); St. Matthew Isl., August 11, 1891, *James M. Macoun* (Hb. U.S. 249296); St. Paul Isl., August 3, 1891, *idem* (Hb. Can. 20478); St. Paul Isl., dampish banks, July 13, 1896, *idem* (Hb. Can. 20479); St. Paul Isl., grassy banks, July 1897, *idem* (Hb. Can. 20481; labeled "*Taraxacum Chamissonis*, Greene typical" in Greene's own handwriting); St. Paul Isl., June 23–August 7, 1914, *idem* (Hb. Can. 94004); Kodiak Isl., crevices of rocks, May 31, 1897, *idem* (Hb. Can. 16754); Hall Isl., crevices of rocks, August 11, 1891, *idem* (Hb. Can. 20621); Unalaska, July 4, 1896, *idem* (Hb. Can. 16755; labeled typical *T. Chamissonis* by E. L. Greene); Valley of Alatna River, about 15 miles above its mouth, July 20, 1901, *W. C. Mendenhall* (Hb. U.S. 377350); St. Paul Isl., August 4, 1891, *C. Hart Merriam* (Hb. U.S. 424071); Kenai, June 9, 1901, *H. P. Nielsen* 11 (Hb. U.S. 378436); St. Paul's Island, July 19, 1890, *Wm. Palmer* 304 (Hb. U.S. 327969); Kodiac, July 28, 1904, *C. V. Piper* 4231 (Hb. U.S. 420683); Kenai, August 18–20, 1904, *idem* 4228 (Hb. U.S. 420680); Unga Isl., Shumagin Isls., July 12–14, 1899, *DeAlton Saunders* (Hb. U.S. 376801); Adakh Isl., July 1, 1893, *C. H. Townsend* (Hb. U.S. 219332); St. Paul Isl., August 14, 1895, *True* and *Prentiss* 82 (Hb. U.S. 231549); Tuksuk Channel, vicinity of Port Clarence, rocky banks, August 5, 1901, *F. A. Walpole* 1746 (Hb. U.S. 378852); Cape Espenberg, lat. 66° 38′ N., long. 163° 46′ W., July 28, 1894, *James T. White* (Hb. U.S. 270305); St. Lawrence Isl., August 27, 1894, *idem* (Hb. U.S. 270328).

Yukon: Canyon of the Upper Liard River, lat. 60°, June 26, 1887, *Dawson* (Hb. Can. 15119; type of *Taraxacum lacerum* Greene); Coral Creek Hill, Yukon River, June 29, 1893, *Frederick Funston* 101 (Hb. U.S. 370774); Herschell Isl., lat. 69° 35′ N., long. 139° W., August 1914, *Frits Johansen* 233 (Hb. Can. 98717; Hb. Field 483379); Five Finger Rapids, July 4, 1899, *J. B. Tarleton* 72 (Hb. U.S. 391518).

British Columbia: Mt. McLean, near Lillooet, alt. 7000 ft., July 29, 1916, *J. N. Macoun* (Hb. Can. no. 98692 in Hb. Field, 483391); Mt. McLean, alt. 6500 ft., July 29, 1916, *idem* (Hb. Can. no. 98693 in Hb. Field, 483392); Mt. McLean, alt. 6300 ft., July 29, 1916, *idem* (Hb. Can. 98694); Mt. McLean, alt. 6000 ft., July 29, 1916, *idem* (Hb. Can. 98695); Mt. McLean, along irrigation ditch, alt. 5000 ft., July 3, 1916, *idem* (Hb. Can. no. 98696 in Hb. Field, 483393); Mt. McLean, alt. 6500 ft., July 22, 1916, *idem* (Hb. Can. no. 98697 in Hb. Field, 483394); Mt. McLean, alt. 5500 ft., July 19, 1916, *idem* (Hb. Can. no. 98699 in Hb. Field, 483395); Whipsaw Creek, west of

Princeton, July 24, 1905, *idem* (Hb. Can. 77000); Yale, grassy slopes, May 17, 1889, *John Macoun* (Hb. Can. 15120); Spence's Bridge, damp grassy places, May 28, 1889, *idem* (Hb. Can. 15130); Fraser River, west of, damp grassy places, June 10, 1875, *idem* (Hb. Can. 15121); Kicking Horse Lake, Rocky Mts., July 18, 1885, *idem* (Hb. U.S. 219795).

Alberta: Jasper Park, at Shovel Pass, low ground near a brook, alt. 6000–6500 ft., August 20, 1918, *James M. Macoun* (Hb. Can. nos. 98686, 98687, and 98688 in Hb. Field, 483386, 483387, and 483388, respectively); Island Creek, north of Peace River, July 15, 1903, *idem* (Hb. Can. 61240); Bragg's Creek, foothills south of Calgary, July 16, 1897, *John Macoun* (Hb. Can. 22776); Calgary, 3 miles west of, along railroad, June 7, 1897, *idem* (Hb. Can. 22792); Banff, swamps, June 27, 1891, *idem* (Hb. Can. 15127); St. Ann, June 9, 1898, *W. Spreadborough* (Hb. Can. 19744).

Utah: Uintah Mts., above Bear River, alt. 12000 ft., August 1869, *Sereno Watson* 723 (Hb. U.S. 41937); Marysvale, alt. 6000 ft., May 21, 1894, *Marcus E. Jones* 5338 (Hb. U.S. 326832; a very unique specimen with exterior bracts of involucre greatly elongated and almost equal to the interior bracts, the flowering head over 5 cm. wide).

California: Bear Valley, San Bernardino County, alt. 6500 ft., June 3, 1901, *S. B. Parish* 4977 (Hb. U.S. 414859).

Besides the specimens cited, I have examined a number from the locality (Kamchatka; also Bering Island, Commander Islands, etc.) whence LEDEBOUR obtained his type. Most of the material from that vicinity, from the Aleutian Islands, and from Alaska proper, has the outer bracts tending to be rather short, ovate, and notably blackish when dried, with the scarious margins highly distinct. This character is not constant, however, and there are numerous variations seen. South of Alaska, nearly every specimen examined has longer, more lanceolate bracts, which tend to remain pale or dark green when dried. Even here, however, there are some marked exceptions to the rule. Thus, for example, *Standley* 4022 from New Mexico (Hb. U.S. 498416) has the dark, scarious-margined, ovate outer bracts typical of the Alaskan material.

GREEN (Pittonia 4:228. 1901), writing upon *Taraxacum* in North America, named the Bering Sea form *T. Chamissonis*.[7]

[7] While GREENE cited no type, many of the Bering Sea specimens listed (in Hb. Can. and Hb. U.S.) had been examined by him and are labeled *T. Chamissonis* in his own handwriting. As noted, the specimen by *J. M. Macoun* from St. Paul Island (Hb. Can. 20481) had been labeled "typical" by him and may be regarded as being practically type material.

He stated that "its most constant peculiarity is that of a very dark-colored, almost blackish, involucre, of which the outer scales are very broad, strictly erect, and imbricated." Reference to LEDEBOUR'S work, however, shows that this was essentially the form which LEDEBOUR described from Kamchatka as *T. ceratophorum* ("squamis omnibus erectis; exterioribus lato-lanceolatis, nigricantibus" etc.),[8] hence *T. Chamissonis* is to be regarded as typical *T. ceratophorum*.

T. lacerum Greene and *T. mutilum* Greene are plainly mere foliage forms of *T. ceratophorum*. The type sheet of *T. lacerum* (in Hb. Can.) bears four small plants. These are not noticeably different from ordinary *T. ceratophorum* except as to the unique leaves,[9] which consist only "of a linear rachis-like body and a few pairs of divaricate or retrorse subulate-linear or falcate lobes." The bracts are highly ceratophorous. I have not been permitted to examine the type of *T. mutilum* (in Hb. Mo. Bot. Gard.), but an excellent cotype, previously cited, is in the U.S. National Herbarium.[10] This has leaves slightly less reduced than in *T. lacerum*, but bracts practically as corniculate. It is matched very closely by *J. M. Macoun's* plant from Churchill, Hudson Bay (Hb. Can. 79286), and, somewhat less closely, by *White* and *Schuchert* 110 from Baffin Land. The discontinuous distribution indicated by the four collections (*T. lacerum* from northern boundary of British Columbia, *T. mutilum* from Johnson River in Alaska, from along Hudson Bay, and from Baffin Land), suggests that either these forms represent one valid species of highly interrupted range or else they are merely foliage forms of *T.*

[8] Fl. Altaica 4:149. 1833. In his still earlier work (Icon. pl. Fl. Ross. 1:9. 1829), LEDEBOUR gave only an abridged description: "L. anthodii squamis erectis infra apicem longe corniculatis; exterioribus lato-lanceolatis; interioribus lanceolatis foliis runcinato-sinuatis: laciniis inaequalibus; majoribus subtriangularibus. Hab. in Kamtschatka. 4 Fl. Majo, Junio." His accompanying plate (*pl. 34*) is somewhat crude and shows the outer involucre spreading above the middle and consisting of narrowly lanceolate or even linear bracts. Apparently LEDEBOUR himself had noticed this discrepancy; for in his later description in the *Flora Altaica*, not only did he retain the character "lato-lanceolatis" for the outer bracts, but he actually inserted the word "omnibus" to qualify "squamis erectis."

[9] These resemble very closely those figured by HANDEL-MAZZETTI (Monogr. Taraxacum, *pl. 5. fig. 2.* 1907) for *T. balticum*, a species unknown to me.

[10] Indeed, GREENE himself had even written "type" upon the label of this specimen, although in his description he listed Hb. Mo. Bot. Gard. as containing the type.

ceratophorum. Touching this point, a parallel study of *T. lyratum* is very illuminating. In several cases I have seen among material that was positively *T. lyratum* a freakish foliage form that looked superficially just like *T. mutilum*. In fact one of these specimens (*Walpole* 1987, Hb. U.S. 379107) appears to have deceived GREENE, for he had labeled it *T. mutilum*. Inasmuch as true *T. lyratum* is seen thus to produce a similar foliage form at times, and since true *T. ceratophorum* is known to be present wherever *T. mutilum* or *T. lacerum* has been collected, there seems to be no reason for considering either *T. mutilum* or *T. lacerum* distinct from *T. ceratophorum*. At the most they evidently can rank no higher than mere forms or varieties.[11]

Many older specimens have been determined in herbaria, some by ASA GRAY, as *T. montanum* Nutt. (non Mey. et DC.), a species cited by NUTTALL from "on the banks of the Platte, in subsaline situations toward the Rocky Mountains, and in the highest valleys of the Colorado of the West." This name was retained by WOOTON and STANDLEY (Contr. U.S. Nat. Herb. **19**:627. 1915) notwithstanding the validity of the previous name *T. montanum* (Mey.) DC. RYDBERG (Fl. Rocky Mts. 1035. 1917), however, recognizing the impropriety of retaining NUTTALL'S duplicating name, created the new and similar name (*Leontodon*) *monticola*, which thus is directly equivalent by synonomy with NUTTALL'S species. Even if NUTTALL'S species had been taxonomically worthy, however, which it was not, RYDBERG'S new name for it would be invalid, as GREENE (*loc. cit.*) had already created the name *T. dumetorum* for material which came from the same region and which did not specifically differ.[12] Obviously GREENE'S name would have

[11] It may be noted, however, that HANDEL-MAZZETTI (Monogr. Taraxacum 87. *pl. 5. fig. 2.* 1907) separates an apparently corresponding form of Europe, *T. balticum*, from the broader leaved *T. paludosum* (cf. footnote 9).

[12] WOOTON and STANDLEY, and also RYDBERG do in fact present *T. dumetorum*, which they have sought to differentiate as a separate species. I have examined all the types (in Hb. Greene) and other specimens cited for *T. dumetorum* by GREENE, and can find no differences other than those that can be proved to be field variations, or that would pass with the great majority of taxonomists as typifying merely inconstant forms. NUTTALL'S description, "caliculum biserial, short and appressed, the scales ovate or lanceolate, with broad membranaceous margins; sepals not corniculate, about twelve" shows that his plant was the form later treated by HANDEL-MAZZETTI as *T. lapponicum* Kihlm. In NUTTALL'S plant the bracts were thus not corniculate, whereas in typical *T. dumetorum* cornicula are present. These distinctions, however, do not appear to be of any value specifically.

preference. Yet even here we are confronted with difficulty, since the *T. dumetorum* type specimens (from Dale Creek, Wyoming, n Hb. Greene) are clearly a mere form or variety of true *T. ceratophorum*. Indeed, an additional "quite typical" specimen cited by GREENE (*Williams* 434) had once been listed by RYDBERG himself (Fl. Montana 484. 1900) as *T. ceratophorum*. Why he later abandoned the name (vide Rydb., Fl. Rocky Mts. 1034–1035. 1917) is not clear. As already stated, the American specimens from points south of Alaska (as also many from Alaska itself) tend to have external bracts somewhat different from those of Bering Sea (that is, typical) material. These exterior bracts vary from dark to light, from short to long, from ovate to lanceolate, from corniculate or widely dilated-bifid at apex to ecorniculate and acute, from appressed to spreading.[13] Occasionally they are as long as the inner bracts. Sometimes both sets of bracts are apically dilated, sometimes only the outer or inner set. Viewed in the light of these facts, *T. dumetorum* is seen to be synonymous with *T. ceratophorum*.

HANDEL-MAZZETTI (*loc. cit.* 73), in dealing with *T. ceratophorum*, makes a singular segregation of specimens under the separate binomial *T. lapponicum* Kihlm. The range given is essentially the same as recognized by him for *T. ceratophorum*. The chief diagnostic distinction relied upon appears to be the ecorniculate character of the bracts. It is with reluctance that I am compelled to reject his treatment.[14] The species concept and "species sense" of one who, like HANDEL-MAZZETTI, has surveyed the entire genus for all the regions of the world, are naturally and very properly entitled to high respect, but the variations in the corniculate character of the bracts are so great in North American specimens as to render illogical and really impossible any such differentiation (cf. footnote 12). It does not also appear that we even have two parallel series, connected, as stated by HANDEL-MAZZETTI, with each other by numerous intermediate

[13] In one specimen from the type locality of *T. ceratophorum* (*C. Wright*, Petropaulovski, Kamchatka, 1853–1856, Hb. U.S. 424073), the outer bracts are lanceolate and their margins are scarious only to a very slight degree.

[14] At least as to North American plants. As to the status of *T. lapponicum* Kihlm. in Europe, I have seen too few specimens to judge accurately.

forms.[15] The *lapponicum* form is much less abundant and appears to be merely an offshoot from *T. ceratophorum*. Sometimes, however, especially in the northeastern part of the continent, it passes into *T. vulgare*.[16] FERNALD and ROBINSON (Gray's *Manual*, ed. 7. 865. 1908) evidently included some of these transitional forms in their *T. officinale* var. *palustre* Blytt, from "eastern Quebec to Connecticut." At the time true *T. ceratophorum* was unknown to them from New England (cf. FERNALD, Rhodora 4:155. 1902), but since then it has been discovered by PEASE (Rhodora 19:111 and 221. 1917) in New Hampshire; and many years before a specimen was collected by ROBBINS.[17] The true *T. officinale* var. *palustre* (*T. paludosum* [Scop.] Schlecht.) is not cited for North America by HANDEL-MAZZETTI.[18]

T. leiospermum Rydb., from Colorado, is found to differ from the ordinary *T. ceratophorum* merely in having slender ecorniculate bracts and a slightly greenish tint to the brown, less muricate achenes. In HANDEL-MAZZETTI's treatment *T. leiospermum* would belong, more precisely, with *T. lapponicum*. Of all the many specimens that I have studied, I have found no other specimen exactly matching RYDBERG's type (in Hb. N.Y.) in the smoothness and color of the achenes. My failure in this respect suggests that the type was merely one of the excessively numerous forms conspicuous in this genus, which apparently often are

[15] "In der ganzen Zone der Gebirge des westlichen Nordamerika ist *T. ceratophorum* mit *T. lapponicum* durch zahlreiche Formen verbunden, die in den Merkmalen der Hüllblättchen Zwischenstellungen einnehmen," *loc. cit.* 66.

[16] HANDEL-MAZZETTI (*loc. cit.* 84) gives an exhaustive treatment of numerous forms intermediate between *T. vulgare* and *T. paludosum*, the latter being a species very close to *T. lapponicum*. He cites none for America, however.

[17] I have not seen this specimen. It was found in the herbarium at Berlin by HANDEL-MAZZETTI, and was determined by him as *T. lapponicum*.

[18] At various times some of our foremost American botanists have used the names *palustre* and *alpinum* for American specimens of *T. ceratophorum*. The real *T. paludosum* (Scop.) Schlecht and *T. alpinum* (Hoppe) Heg. and Heer, dating back originally to 1772 and 1821 respectively, are not given by HANDEL-MAZZETTI for North America. While I have been unable to examine enough European material to permit of definite conclusions, it would seem that the two species are too close together. In any case, it appears certain that if American forms of *T. ceratophorum* with ecorniculate bracts are to be segregated, they must be referred to *T. paludosum* or *T. alpinum*, rather than to the more recent *T. lapponicum*.

perpetuated here and there through parthenogenetic reproduction.[19]

Taraxacum lividum Heller (exclud. synon. Waldst. et Kit.) is seen, from the specimens cited (*A. A.* and *E. C. Heller* 3642), to be likewise a form of *T. ceratophorum*. Most of the bracts are ecorniculate, thus placing the plants, in HANDEL-MAZZETTI's treatment (*loc. cit.* 74), with *T. lapponicum*.

3. TARAXACUM ERIOPHORUM Rydb., Fl. Montana, Mem. New York Bot. Gard. 1:454. 1900 (non Schott ex Tchihatcheff, Asie mineure 3^2:372. 1860; nomen nudum quod = *T. syriacum* Boiss., fide Handel-Mazz., Monograph. Taraxacum 162. 1907); *T. ovinum* Greene, Pittonia 4:229. 1901; *T. angustifolium* Greene, *loc. cit.*; *T. ammophilum* Nels. ex Greene, *loc. cit.* 233. *L. eriophorum* Rydb., Fl. Rocky Mts. 1035. 1917, *L. angustifolium* Rydb. *loc. cit.*; *L. ammophilum* Rydb., *loc. cit.* Pl. XXXIII.

Herba polymorpha, nunc pumila et rosulata (forma descriptionis orig.), nunc robustior, 3–8 (etiam –30) cm. alta. Radix et folia et scapi eis *T. ceratophori* non conspicue dissimiles, foliis autem saepius membranaceis et pallidis, rarius profunde pinnatifidis, iuvenilibus raro longe lanuginosis versus basim. Capitula 1.5–2.5 cm. alta et paulo latiora. Involucrum pallidum vel atroviride. Involucri foliola plerumque ecorniculata vel rarissime ad apicem dilatato-corniculata et plus minusve atrata, exteriora adpressa vel minime patentia, interiorum longitudinis 0.2–0.6 aequantia, 4–15 mm. longa, margine plus minusve distincte decolorato. Flores vivi ad anthesin non observati. Achaenia 4–5 mm. longa, rufa rufopurpureave, supra tuberculis angustis vel spinulis dense obsita, saepe acute tetragona, in cuspidem crassam vel angustam, et brevem vel quartae parti totius fructus aequantem cuneate attenuata. Rostrum tenue, achaenio paulo vel multo longius. Pappus albus, 4–8 mm. longus.

DISTRIBUTION.—Alberta to Wyoming; a form with highly corniculate bracts occurs in Alaska.

SPECIMENS EXAMINED.—Alberta: Morley, meadows, etc., June 12, 1885, *John Macoun* (Hb. Can. 15117); Laggan, June 28, 1905, *idem* (Hb. Can. 65618 and 65619); Waterton Lake, Sheep Mt., July 31, 1895, *idem* (Hb. Can. 11711, type of *T. ovinum* Greene; Hb. Greene 48435).

[19] Concerning the fixation of new colors in *Taraxacum* achene coats through the operation of parthenogenesis, cf. footnote 24.

Alaska: Vicinity of Port Clarence, gravel flats near beach, Teller Reindeer Station, September 3, 1901, *F. A. Walpole* 1980 (Hb. U.S. 379098).

British Columbia: Kicking Horse Lake, Rocky Mountains, springy places, July 20, 1885, *John Macoun* (Hb. Can. 15128; Hb. Field 227895).

Montana: Sheridan, in 1892, *Mrs. L. A. Fitch* (Hb. Mont. Agric. Exper. Sta.; type); Anaconda, mountain swales, alt. 6000 ft., May 20, 1906, *J. W. Blankinship* 723 (Hb. Can. 73794; Hb. Field 225568; Hb. U.S. 541188).

Wyoming: Dale Creek, July 1, 1896, *Edward L. Greene* (Hb. Greene 48449, 48450, and 48451; the three type sheets of *T. angustifolium* Greene); Pole Creek, June 2, 1894, *Aven Nelson* 109 (Hb. U.S. 284425); Horse Creek, June 9, 1894, *idem* 205 (Hb. Field 432099; Hb. U.S. 284424); Sand Creek, Albany Co., May 31–June 1, 1900, *idem* 6987 (Hb. Greene 48427, type of *T. ammophilum* Nelson ex Greene; Hb. U.S. 433375); Sand Creek, Albany Co., June 1, 1900, *idem* 6988 ex parte (Hb. U.S. 433376).

The specimens originally distributed by NELSON (no. 6987) as *T. ammophilum* are rather small, averaging mostly under 1 dm. in height, and are of a pallid, somewhat glaucous appearance. Their achenes, when mature, are distinctly reddish, as in *T. laevigatum*. The involucral bracts are almost entirely without dilations at the apex. Except for the achenes, the plants match perfectly some plants considered by HANDEL-MAZZETTI as *T. lapponicum* Kihlm., but regarded by myself as a form or variety of *T. ceratophorum*. They are in no way referable to the European *T. laevigatum*, as suspected by HANDEL-MAZZETTI (*loc. cit.* 110), who appears never to have seen NELSON'S specimens.

Some of the material examined is darker green, but otherwise identical. *Blankinship* 723 from Montana, consisting mostly of immature specimens, is an example of this. The Blankinship plants are particularly instructive, further, in showing the aspect of immature and dwarfed plants. Some of these (for example, Hb. Field 225568) match exactly RYDBERG'S three tiny immature type specimens of *T. eriophorum* (in Hb. Mont. Agric. Exper. Sta.).[20] RYDBERG did not describe the achenes, since there were no mature ones present.[21] The immature achenes of the Blankinship collection are brown, as in RYDBERG'S type material, but the

[20] BLANKINSHIP'S plants were collected at Anaconda, a distance of only 55 miles (90 km.) from Sheridan, whence the type of *T. eriophorum* came.

[21] The name *eriophorum* alluded to the brown hairs found on the small type plants, but this character is entirely inconstant in this species and has no real taxonomic value.

nearly mature ones (for example, Hb. U.S. 541188) are distinctly reddish. Thus RYDBERG's type plants are seen to be connected perfectly with the type material of *T. ammophilum*, and, from priority, the name *T. eriophorum* must have the preference.

The type material of *T. ovinum* Greene, from Alberta, consists of several small, more or less dwarfed and immature specimens. The achenes in the oldest head found (in Hb. Can.), while not yet very reddish, have the acutely tetrangular shape that I have observed in numerous other mature specimens of *T. eriophorum*. The involucre, although sometimes duplicated by *T. ceratophorum*, is more typical of *T. eriophorum*, and there remains no doubt that *T. ovinum* is purely synonymous with *T. eriophorum*.

T. angustifolium Greene was founded upon three specimens from Dale Creek, Wyoming. The leaves and scapes are much better developed than in *T. ovinum*, the scapes reaching a height of over 2.5 dm.; but the technical characters of the head are essentially the same. Moreover, the numerous mature achenes are definitely reddish in color. GREENE (*loc. cit.* 232) termed their color "chestnut brown," but inaccurately so, for the color is fully as reddish as in many genuine specimens of the red-achened *T. laevigatum*. The leaves are rather long, slender, and graceful, but certainly do not serve to separate the plants specifically from true *T. eriophorum*.[22]

HANDEL-MAZZETTI (*loc. cit.*) has omitted *T. eriophorum* Rydb. entirely from his monograph, and it is evident that he was entirely unfamiliar with it. The species is closely parallel with *T. ceratophorum*, from which it differs in having red achenes and in having the bracts much more often slender and without dilated tips. One might wonder whether it may be only a form of *T. ceratophorum* in which the achenes are red. Various investigators have shown that apogamy or parthenogenesis is frequent in *Taraxacum*.[23] SCHKORBATOW (Entwickelungsgeschichtliche Stud. an *Taraxacum officinale* Wigg., Bot. Institut. Charkow, p. 50. 1910) also states

[22] Almost the exact counterpart to this foliage is sometimes observed in a form of *T. ceratophorum* (for example, *Mendenhall*, Valley of Alatna River, Hb. U.S. 377350).

[23] For references to the experiments and observations of RAUNKIAER, MURBECK, JUUL, and others, see IKENO, Ber. Deutsch. Bot. Gesells. 28:394. 1910.

that various colors of achenes may thus become fixed and hereditary.[24] Whether, however, the colors will remain fixed in the achenes of all the plants of a locally generated race upon a recurrence of normal fertilization (with attendant lapse of apogamy) is doubtful. Surely subsequent cross-pollination with specimens from the antecedent stock might be expected to occur and to result, at times, in a repetition of the former achene color. In any case, the observable tendency of the achenes of *T. eriophorum* to be more sharply tetragonal and of the bracts to be undilated at the apex in a much higher percentage of specimens, makes it seem that *T. eriophorum* is not a red-fruited variety of *T. ceratophorum*, but is rather a distinct species.

The Alaskan specimen by *Walpole* (no. 1980, Hb. U.S. 379098) has slender elongate leaves, much as in the types of *T. angustifolium*, and its achenes are bright red. The involucral bracts, however, especially the inner ones, are exceedingly corniculate, much as in the extremely corniculate forms of *T. ceratophorum*.[25]

4. TARAXACUM VULGARE (Lam.) Schrank, Primit. Fl. Salisburg. 193. 1792; *Leontodon Taraxacum* Linn., Sp. Pl. **2**:798. 1753 (diagnose incompl. fide Handel-Mazz.); Pollich, Hist. plant. Palatin. **2**:379. 1777; *L. vulgare* Lamarck, Fl. Françoise **2**:113. 1778; *T. officinale* Weber, Prim. Pl. Holst. 56. 1780; Roth, Tentam. Fl. Germ. **2**²:147. 1793; *T. Dens-leonis* Desf., Fl. Atlant. **2**:228. 1800 (fide Indicis Kew., locum cit. non vidi); *T. latilobum* DC., Prodr. **7**:146. 1838; *T. mexicanum* DC., *loc. cit.*; *T. officinale* var. *palustre* Fernald and Robinson, Gray's Man., ed. 7, p. 865. 1908 (forsan non [Smith] Blytt, Bentham, et al.); *T. paradoxum* Somes, Amer. Botanist **15**:27. 1909; *L. latilobum* Britton, Britt. and Brown Ill. Fl. N. Amer., ed. 2, **3**:315, *fig. 4063*. 1913; *T. minus*

[24] "In der Natur findet man verschiedene Farben-Schattierungen an den *Taraxacum*-Früchten, von dunkelbraun bis hellgrünlich: die ausgesprochenen Färbungen in typischen Modificationen genommen (rein hellgrün und rein dunkelbraun) werden als solche durch Vererbung fixirt." (L. SCHKORBATOW, *loc. cit.* For English summary of SCHKORBATOW's work, see CHAMBERLAIN, BOT. GAZ. **52**:167. 1911.)

[25] To those who accept HANDEL-MAZZETTI's differentiation of North American material between *T. ceratophorum* and *T. lapponicum*, this specimen will appear correspondingly distinct from *T. eriophorum*. I have found no such intermediate forms in *T. eriophorum*, respecting dilations of the bract tips, as are abundant in *T. ceratophorum*. Nevertheless, there seems insufficient evidence at hand to warrant proposing *Walpole's* 1980 as the type of a new species.

Lon. et var. *subscaposum* Lunell (ex synon. *L. Taraxacum* Britton, etc.), Amer. Midl. Nat. 5:31. 1917; *L. mexicanum* Rydb., Fl. Rocky Mts. 1034. 1917.

Herba plerumque maiuscula, 5–50 cm. (rarissime "—1.20 m.") alta. Radix crassa, simplex vel multiceps, fusce corticata, collo vix squamato, large lanigero vel raro glabro. Folia nunc terrae adpressa, nunc suberecta, viridia, plerumque infra et in nervo medio sparse pilosa vel rarius glaberrima, plerumque ampla, plus minusve oblanceolata (7 mm. —15 cm. lata), acuta vel obtusa, versus basim brevius longiusve angustata, rarius large dentata tantum, plerumque autem variis modis, interdum usque ad nervum medium, runcinato-incisa, lobis latius angustiusve triangularibus vel rarius linearibus, integris vel dentato-fissis, recurvis, saepe lobulis minoribus interiectis, lobo terminali plerumque maiore. Scapi numerosi vel raro singuli, erecti vel adscendentes, crassi (2–7 mm.), florendi tempore sub capitulo saltem longe lanigeri, denique raro glabri, floriferi foliis ±aequilongi, rarius multo breviores vel multo longiores. Capitula magna (solum in speciminibus depauperatis parva), circum 2–2.5 cm. longa et aperta latitudine multo maiore. Involucri foliola numerosa, utriusque seriei ±15–20, griseo-viridia, raro atrata, interdum leviter pruinosa, ecorniculata vel raro corniculis parvis vel rarissime maioribus instructa, linea dorsali fusca nulla. Exterioris seriei foliola interioribus vix latiora, sed paulo breviora, inter se fere aequilonga, iam in alabastris adultioribus supra basim reflexa, vel raro patula vel unum alterumve eorum vel rarissime plurima semper erecta, linearia (1.3–3 mm. lata et 12–14 mm. longa) vel rarissime latiora, margine raro indistincte decolorato. Flores numerosissimi, lutei vel raro subpallidiores, involucro circum 5–10 mm. longiores. Achaenia parva, 3–4 mm. longa, pallide griseo- vel olivaceo-brunnea, supra tuberculis mediocribus longioribusve dense obsita, in cuspidem cylindricam longiusculam et tenuem vel brevissimam et crassam, totius fructus sextam vel rarius tertiam fere partem metientem abruptissime contracta. Rostrum tenue, achaenio duplo vel plus triplo longius. Pappus albus, 6–8 mm. longus, rostro brevior.

Distribution.—Labrador and North Carolina to Alaska, California, and Mexico, and elsewhere almost throughout the world; indigenous (fide Handel-Mazz.) in meadows of Europe and Western Asia.

SPECIMENS EXAMINED.—Labrador: Rama, August 20–24, 1897, *J. D. Sornberger* 64*x* (Hb. U.S. 411050; a form with the leaves lanceolate to spatulate and not deeply incised, some of them merely denticulate).

Newfoundland: Hermitage Bay, vicinity of Balena, June 16, 1903, *William Palmer* 1365 (Hb. U.S. 492202).

Quebec: Mt. Albert, Gaspe County, by alpine brooks or in crevices of wet hornblende schist, alt. 600–1075 m., July 20, 1906, *Fernald* and *Collins* 263 (Hb. U.S. 606098); Mt. Albert, Gaspe County, meadows and fields, also on mountains, August 19, 1882, *John Macoun* (Hb. Can. 15113); Salt Lake, Anticosti Isl., pastures and fields, August 11, 1883, *idem* (Hb. Can. 15105); Orono and vicinity, fields, September 1890, *F. L.* and *LeRoy H. Harvey* 579 (Hb. U.S. 606242); Orono, June 2, 1897, *P. L. Ricker* 233 (Hb. U.S. 414356); St. Francis River, at Boundary Lake, August 14, 1902, *W. W. Eggleston* (Hb. U.S. 492531).

Massachusetts: Middleboro, May 14, 1901, *Joseph Murdoch* (Hb. Field 471888); Middleboro, May 14, 1901, *Richard Murdoch* (Hb. Field 472180).

Rhode Island: Cumberland, railroad embankment, May 9, 1900, *E. B. Chamberlain* 68 (Hb. U.S. 491069).

New York: Chemung County, roadsides and fields, May 19, 1893, *T. F. Lucy* 14529 (Hb. Field 5306); Cold Spring Harbor, Long Island, waste places, August, 1903, *H. N. Whitford* 20 (Hb. Field 144122).

Pennsylvania: Westtown Farm, Chester County, May 26, 1905, *S. P. Hadley* 1 (Hb. U.S. 646339); Ephrata, vicinity of, May 14, 1900, *A. A. Heller* (Hb. Field 430006; Hb. U.S. 407015); Conestoga Creek, east of Lancaster, *idem*, April 28, 1900 (Hb. U.S. 407016; form with finely divided leaves); Conestoga Creek, Lancaster, May 2, 1890, *John K. Small* (Hb. Field 168088 and 168089); Harrisburg, June, 1887, *idem* (Hb. Field 168174); Conewago, vicinity of, May 14, 1891 (Hb. Field 167805 and 167810).

District of Columbia: without locality, in 1863, herb. M. S. Bebb (Hb. Field 17549).

Virginia: Louden County, August 1888, *Jesse H. Holmes* (Hb. U.S. 41946 and 41948); Chatham Hill Gap, Walker Mountain, Smyth County, alt. 3000 ft., June 13, 1892, *John K. Small* (Hb. Field 390271); White Top Mountain, Smyth County, alt. 4000–5000 ft., May 28–29, 1892 (Hb. Field 390272).

West Virginia: Pickens, June 24, 1908, *Huron H. Smith* 1364 (Hb. Field 241895).

North Carolina: Roan Mountain, September 1, 1902, *W. A. Cannon* 223 (Hb. U.S. 510188).

Ontario: Kingston, May 27, 1897, *J. Fowler* (Hb. Field 83469); Kingston, May 29, 1895, *idem* (Hb. U.S. 249777).

Ohio: Dayton, abundant and troublesome as a weed, May 25, 1904, *J. Lane Reed* (Hb. U.S. 444728 and 444729; a gigantic form escaped from cultivation, the leaves becoming, before end of fruiting period, over 4 dm. long and the scapes 7.75 dm. long); Chillicothe, in 1885, *H. T. Safford* 12 (Hb. U.S. 515462).

Michigan: Schoolcraft, uncleared ground, June 11, 1903, *A. B. Burgess* 129 (Hb. Field 141460).

Indiana: Mattsville, vicinity of, in open ground, May 10, 1892, *Guy Wilson* 19 (Hb. U.S. 228418); Mishawaka, June 1891, *E. B. Uline* (Hb. Chi. 260181).

Wisconsin: Green Bay, April, *J. H. Schuette* (Hb. Field 377994); Brown County, in yard, without date, *idem* (Hb. Field 377995).

Illinois: Evanston, dry field, July 4, 1919, *Earl E. Sherff* 3087 (Hb. Field 484462 and 484463), and in rich woods, July 4, 1919, *idem* 3088 and 3090 (Hb. Field 484464, 484465 and 484468, 484469 respectively); Urbana, open thicket, May 28, 1907, *Frank C. Gates* "1561:3" (Hb. U. S. 649050).

Minnesota: Fort Snelling, May–June, 1890, *E. A. Mearns* 161 (Hb. U.S. 649285 and 649286).

Iowa: Decatur County, pastures and waysides, common, May 28, 1896, *T. J.* and *M. F. L. Fitzpatrick* (Hb. Field 123803).

Missouri: Vulcan, railway tracks, May 8, 1908, *Huron H. Smith* 449 (Hb. Field 240920).

North Dakota: Grand Forks, vicinity of, in 1894, *C. A. Egebretson* 43 (Hb. Chi. 351987).

South Dakota: Mayo, meadows, June 20, 1914, *W. H. Over* 1828 (Hb. U.S. 582845); Rapid City, alt. 3700 ft., June 25, 1892, *Per Axel Rydberg* 846 (Hb. U.S. 211334).

Nebraska: Lincoln, May 10, 1886, *T. A. Williams* (Hb. U.S. 750371).

Kansas: Riley County, grassland, in 1896, *J. B. Norton* 748 (Hb. U.S. 353535).

Alberta: Jasper Park, Cabin Creek near Jasper, roadsides, June 15, 1918, *James M. Macoun* (Hb. Can. no. 98691 in Hb. Field, 483390).

Wyoming: Crow Creek, Albany County, moist banks, July 8, 1903, *Aven Nelson* 8905 (Hb. U.S. 581938); Yellowstone National Park, October 8, 1902, *Edgar A. Mearns* 4769 (Hb. U.S. 488386).

Colorado: Norwood Hill, San Miguel County, moist river banks, alt. 7000 ft., August 17, 1912, *Ernest P. Walker* 488 (Hb. U.S. 544606); Ouray, July 24, 1897, *C. L. Shear* 4102 (Hb. U.S. 858239).

New Mexico: Las Vegas, May 19, 1909, *T. D. A. Cockerell* (Hb. U.S. 660047); Rio Arriba County, hills south of Tierra Amarilla, alt. 2300 m., April 18–May 25, 1911, *W. W. Eggleston* 6545 (Hb. U.S. 660765); Tierra Amarilla, alt. 2280 m., April 18–May 25, 1911, *idem* 6594 (Hb. U.S. 660810); Raton, in streets, alt. 2100–2380 m., June 21–22, 1911, *Paul C. Standley* 6305 (Hb. U.S. 685335); Chama, vicinity of, along river, alt. 2380–2850 m., July 8, 1911, *idem* 6589 (Hb. U.S. 685611).

Utah: Milford, wet ground, June 4, 1902, *Leslie N. Goodding* 1039 (Hb. U.S. 485541); Provo, Wasatch Mts., June 16, 1902, *idem* 1156 (Hb. Field 215750); Big Cottonwood Canyon, below Silver Lake, June 29, 1905, *Rydberg* and *Carlton* 6455 (Hb. U.S. 508591); Wasatch Mts., abundant on plateau east of Ephraim Canyon, alt. 2900 m., August 14, 1907, *Ivar Tidestrom* 230

(Hb. U.S. 506794); Salt Lake City, alt. 5000 ft., May, 1869, *Sereno Watson* 722 (Hb. U.S. 41950).

Idaho: Pine, moist flat lands, August 16, 1910, *J. Francis Macbride* 619 (Hb. U.S. 542442); New Plymouth, "a terrible pest in lawns," July 14, 1910, *idem* 711 (Hb. Field 292597; Hb. U.S. 542478); Nez Perces County, along Hatwai Creek, April 24, 1892, *J. H. Sandberg* 42 (Hb. U.S. 243000); Hailey, common in empty lots, in 1909, *Woods* and *Tidestrom* 2762 (Hb. U.S.).

Nevada: Battle Mt., alt. 1350 m., July 23, 1913, *Albert E. Hitchcock* 626 (Hb. U.S. 765964); Jarbidge, along brook, July 12, 1912, *Nelson* and *Macbride* 2048 (Hb. U.S. 544856).

Alaska: Sitka, June 14-17, 1899, *Coville* and *Kearney* 804 (Hb. U.S. 376697); Wrangell, grassy hillside, May 6, 1915, Mr. and Mrs. *Ernest P. Walker* 617 (Hb. Field 466422); Wrangell, grassy slope, May 8, 1915, *iidem* 631 (Hb. Field 466435).

British Columbia: Oak Bay, vicinity of Sidney, Vancouver Isl., roadsides, April 22, 1913, *John Macoun* (Hb. Can. no. 98700 in Hb. Field, 483380).

Washington: Waitsburg, April 14, 1897, *Robt. M. Horner* 319 (Hb. U.S. 318829).

Oregon: Keno, alt. 4000 ft., May 9, 1898, *Elmer I. Applegate* 2015 (Hb U.S. 361604); Umatilla National Forest, alt. 4300 ft., June 11, 1912, *C. L. Keithley* (Hb. U.S. 583213); Cottonwood Canyon, Malheur County, alt. 750 m., May 20, 1896, *John B. Leiberg* 2073 (Hb. U.S. 276280).

California: Mt. Shasta, north side of, alt. 5000–10,000 ft., June 15–30, 1897, *H. E. Brown* 442 (Hb. Field 412772); Amador County, March 23, 1896, *George Hansen* 1550 (Hb. Greene 48461).

Chihuahua: Chihuahua, vicinity of, alt. about 1300 m., June 5–10, 1908, *Edward Palmer* 353 (Hb. U.S. 573818).

Coahuila and Nuevo Leon: without locality, February–October, 1880, *idem* 761 (Hb. U.S. 41955).

San Luis Potosi: Alvarez, July 13–23, 1904, *idem* 180 (Hb. U.S. 471047).

Queretaro: Without locality, in 1910–13, *Agniel* 10535 (Hb. Field 484882).

Vera Cruz: Boca del Monte, March 13, 1894, *E. W. Nelson* 226 (Hb. U.S. 252392 *pro parte*); Las Vigas, June, 1893, *idem* 22 sub nomen *Senecio* (Hb. U.S. 252058).

Puebla: Chalchicomula, vicinity of, alt. 8000–8400 ft., March 15, 1894 *idem* 237 (Hb. U.S. 252392 *pro parte*).

Mt. Orizaba: without precise locality, July 25–26, 1901, *Rose* and *Hay* 5722 (Hb. U.S. 395506).

Mt. Popocatepetl: without precise locality, August 7–8, 1901, *iidem* 6067 (Hb. U.S. 395872).

Michoacan: Morelia, in streets, November, 1889, *Alfredo Duges* (Hb. U.S. 41956).

Mexico, civitate non cit.: *Berlandier* 849 (Hb. Boiss., cotype of *T. mexicanum DC.*).

Bermuda Isls.: Flatts, roadsides, August 16, 1913, *F. S. Collins* 314 (Hb. Field 464861); Agar's Isl., "not abundant," December 4, 1915, *idem* 430 (Hb. Field 464906).

Jamaica: Cinchona, alt. 4900 ft., in 1910, *Wm. Harris* 10926 (Hb. Field 294859).

As previously stated, *T. vulgare* tends to pass into *T. ceratophorum* in the northeastern part of North America. The *T. officinale* var. *palustre* of GRAY'S *Manual* (ed. 7, p. 865, *fig. 1015*. 1908) includes some of these transitional forms; so also does *T. latilobum* DC., collected originally in Newfoundland ("invol. squamis ecorniculatis, exter. patulo-reflexis . . . . proxime ad Dentem-leonis accedit," —DC., *loc. cit.*). *Murdoch* 1624, from Massachusetts (Hb. Field 470264) is typical of the GRAY'S *Manual* illustration, and yet is easily recognized as being true *T. vulgare*. *Fernald* and *Collins* 263 (Hb. U.S. 606098) from Quebec has the involucres fairly typical of *T. vulgare*, but in general habit it approaches *T. ceratophorum;* in fact, it was originally under the latter name. *Sornberger* 64*x* (Hb. U.S. 411050) from Labrador is still another form of *T. vulgare*. Its involucres are of the *T. vulgare* kind; but the foliage exactly matches that of *Fernald's* Grand River plant of Quebec (Hb. U.S. 605794), a plant that from involucral characters is seen however to be *T. ceratophorum*. Plants collected by *L. M. Turner* at Davie's Inlet, Labrador (Hb. U.S. 222755), have involucres clearly representing *T. vulgare*, but the foliage is very strange and is closer to that of *T. ceratophorum*, although not typical for that species. It seems entirely probable that a number of these intermediate forms are hybrids.

T. mexicanum DC. is retained as a valid species by HANDEL-MAZZETTI, who had seen at least nine specimens of BERLANDIER'S original type material, but I have seen no specimens of *Taraxacum* from Mexico that were not plainly *T. vulgare*. Even the excellent cotype specimen studied (in Hb. Boiss.) matches much of the *T. vulgare* material of the northern United States in foliage, in fruit, and in involucre. Nor does HANDEL-MAZZETTI'S description indicate any truly distinctive characters. Thus, for example, he describes the cusp of the achenes as being long in *T. mexicanum* and short or very short in *T. vulgare*, but there are numerous

specimens of genuine *T. vulgare* from various points all over North America in which the cusps are very long and slender, fully as much so as in any Mexican material studied by me. De Candolle himself was in doubt as to the validity of his species, even confessing that it was too close to *T. dens-leonis* (*T. vulgare*) and was perhaps only a variety.[26] Hemsley (Biol. Centr. Amer. 2:261. 1881) regarded *T. mexicanum* as synonymous with our *T. vulgare* (*T. officinale*), and my own observations are in thorough accord with Hemsley's treatment.

T. paradoxum Somes was admittedly a mere freak form of *T. vulgare*, having the stem foliate with alternate leaves, not scapose. The stems were bifurcate at the top. Lunell's *T. minus subscaposum* was likewise a mere leafy stemmed form ("caulis unifoliatus"). Such a form was not unknown before (cf. D. McAlpine Bot. Atlas 1: *pl. 25, figs. 6, 13b.* 1883).

5. Taraxacum laevigatum (Willd.) DC., Cat. Hort. Monspel. 149. 1813; *Leontodon laevigatus* Willd., Sp. Pl. 3:1546. 1800; *T. erythrospermum* Andrz. in Besser, Enum. Pl. Volhyn., Podol., etc., 75. 1822; *L. erythrospermum* Eichw., Naturhist. Skizze Litth., Volhyn, etc., 150. 1830; *L. erythrospermum* Britton in Britt. and Brown, Ill. Fl. N. Amer. ed. 2., 3:316. *fig. 4064.* 1913; *T. mexicanum* Wooton and Standley, Fl. New Mex. 626. 1915 (non DC.).

Herba subgracilis, 5–30 cm. alta. Radix tenuiuscula, simplex vel pluriceps, fusce corticata, collo foliorum vetustorum fragmentis persistentibus magnus, plerumque pallide brunneis large squamato, longe lanuginoso vel rarius glabro. Folia terrae adpressa vel suberecta, glabra vel infra parce pilosula, lanceolata 0.5–4 (vel serius etiam −11) cm. lata, versus basim plerumque longe angustata, fere semper tota profunde incisa vel variis modis usque ad nervum medium crebre pinnatisecta, lobis latis angustisve, plerumque acutis, integris vel largius et tenuiter dentatis, plus minusve reflexis, interiectis saepe lobulis dentiformibus, lobo terminali lateralibus paulo maiore vel etiam interdum minore. Scapi singuli vel numerosi, subtenues, erecti vel e basi procumbente adscendentes, floriferi foliis breviores vel longiores, serius plus

[26] "Nimis *T. Denti-leonis affine et forte varietas*," DC., *loc. cit.*

minusve elongati. Capitula parva vel mediocria, circum 1–2 cm. longa et paulo latiora. Involucri foliola utriusque seriei circa 11–13, pallidius atriusve griseo-viridia, glauco-pruinosa, ecorniculata vel plerumque corniculis mediocribus vel parvis instructa. Exterioris seriei foliola adpressa, patentia vel e basi patenti recurva, interioribus latiora eorumque longitudinis tertiam vel dimidiam partem vix superantia, infima ceteris breviora, omnia late vel angustius ovata vel e basi ovata triangularia (4–8 mm. longa et 1.5–3 mm. lata), margine membranaceo plerumque distinctissimo. Flores numerosi, involucro 2–4 mm. longiores, citrini vel (in speciminibus pinguibus) *T. vulgaris* floribus paulo tantum pallidiores, extus plerumque griseo vel rubro striati. Achaenia parva, ±3–4 mm. longa, intense rufa, rufopurpurea vel fere atropurpurea, supra tuberculis longis angustis largis obsita, saepe infra quoque rugulosa, in cuspidem anguste linearem longam, totius fructus tertiam vel quartam partem superantem abrupte contracta. Rostrum tenue, achaenio dimidio vel plus duplo longius. Pappus albus, 4–7 mm. longus.

Distribution.—Nova Scotia and Virginia to British Columbia, Idaho, and New Mexico; apparently introduced from Europe, where native, as also (fide Handel-Mazz.) in western Asia and northwestern Africa.

Specimens examined.—Nova Scotia: Yarmouth, *John Macoun*, June 3, 1910 (Hb. Can. 81358).

Massachusetts: Dorchester, May 24, 1903, *John Murdoch, Jr.*, 1304 (Hb. Field 470218); Weston, May 21, 1904, *idem* 1625 (Hb. Field 470265).

Vermont: Vergennes, dry knolls in orchard, May, 28, 1899, *Ezra Brainerd* (Hb. Greene 48444); West Rutland, Twin Mountains, May 31, 1899, *W. W. Eggleston* 1416 (Hb. U.S. 364398); Shrewsbury, May 25, 1902, *idem* 2681 (Hb. U.S. 492337).

Pennsylvania: Rohrerstown, April 21, 1891, *John K. Small* (2 sheets in Hb. Field, 169751 and 169752); vicinity of Conewago, May 14, 1891, *idem* (Hb. Field 167811).

District of Columbia: Washington, university grounds, May 2, 1899, *Edward L. Greene* (Hb. Greene 48443).

Virginia: North Four Mile Run, May 1898, *Ivar Tidestrom* (Hb. Greene 48441).

Michigan: Durand, May 15, 1913, *Edward L. Greene* (Hb. Greene 22276).

Ohio: Sandusky, July 28, 1903, *E. L. Moseley* (Hb. Field 240265).

Illinois: Chicago, in yard, May 1919, *Winnifred Baantjer* (Hb. Field 485106); Urbana, University campus, April 1, 1907, *Frank C. Gates* "1369.3"

(Hb. U.S. 648981); White Heath (Piatt Co.), ballast of railroad, May 4, 1907, *idem* 1432 (Hb. U.S. 649007); Evanston, near walk, July 4, 1919, *Earl E. Sherff* 3089 (Hb. Field 484466 and 484467).

Missouri: Vicinity of Springfield, pastures, August 28, 1911, *Paul C. Standley* 8287 (Hb. U.S. 687249).

Nebraska: Omaha and vicinity, street, August 16, 1905, *Amy C. Lawton* 65 (Hb. Field 103610).

New Mexico: Chama (Rio Arriba Co.), alt. 2380 m., May 26, 1911, *W. W. Eggleston* 6665 (Hb. U.S. 660876).

Alberta: Athabasca Landing, July 28, 1914, *A. S. Hitchcock* 12158 (Hb. U.S. 885176).

Idaho: Coeur d'Alene, abundant in lawns at city limits, August 11, 1913, *Henry J. Rust* 396 (Hb. U.S. 870324).

Wyoming: Yellowstone National Park, June 4, 1902, *Edgar A. Mearns* 939 (Hb. U.S. 486330).

British Columbia: Beavermouth, floodplain of Columbia, alt. 2400 ft., August 18, 1905, *C. H. Shaw* 1149 (Hb. U.S. 622044).

This species should not be confused with *T. laevigatum* A. Gray (Proc. Acad. Phil. 1863:70), which was synonymous with *T. lyratum* (Led.) DC. In recent American literature it has been known as *T. erythrospermum*, but HANDEL-MAZZETTI (Monogr. Taraxacum 109. 1907) has seen WILLDENOW's original specimen of *Leontodon laevigatum* and found that *T. erythrospermum* is purely synonymous with it. BRITTON (*loc. cit.*), familiar only with the name *Taraxacum erythrospermum*, but rejecting the generic name *Taraxacum*, has lately used the name *Leontodon erythrospermum* for this species; but this last combination (made by EICHWALD in 1830) is untenable of course, since under the appellation *Leontodon*, the name *Leontodon laevigatus* antedates it by a number of years. WOOTON and STANDLEY (*loc. cit.*) have confused this species with *T. mexicanum* DC. (*T. vulgare*). From their herbarium determinations and also from their description, "achenes red," it is seen that their plants were purely *T. laevigatum*.

Specifically, *T. laevigatum* is much the most clearly marked and sharply defined of any of our native or introduced North American species of *Taraxacum*.

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SHERFF on TARAXACUM

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SHERFF on TARAXACUM

EXPLANATION OF PLATES XXXI–XXXIII

PLATE XXXI

Taraxacum lyrata

FIGS. *a* and *j* (×0.48). *T. alaskanum* form: *Coville* and *Kearney* 1097, Haenke Isl., Hb. U.S. 376702; figs. *b* and *f* (×0.48), foliage form typical as to LEDEBOUR'S type illustration, but plants less compound at base than figured by LEDEBOUR, *Coville* and *Kearney* 2164, St. Matthew Isl., Hb. U.S. 376718; figs. *c*, *g*, and *i* (×0.63), showing (*c*) foliage form of *T. lyratum* that corresponds to *T. mutilum* form of *T. ceratophorum* and matches type figures of *T. hyperboreum* Dahlst. and *T. curylepium* Dahlst., (*g*) foliage form matching VAHL'S type plate of *T. phymatocarpum*, and (*i*) foliage form of *T. lyratum* more nearly approaching that of LEDEBOUR'S plate, all three from *Walpole* 1791, Alaska, Hb. U.S. 378905; fig. *d* (×0.56), from type sheet of *T. alaskanum*, McIlhenny 111, Alaska, Hb. N.Y.; fig. *e* (×0.52), topotype of *T. alaskanum*, Murdoch, Alaska, Hb. U.S. 424063; fig. *h* (×0.70), foliage form closely matching some of more erect "*T. phymatocarpum*" forms from Greenland, *Knowlton* 142, Arizona, Hb. U.S. 41949; fig. *k* (×0.79), tiny dwarf form of Rocky Mts. (*T. officinale* var. *scopulorum* Gray), *Baker*, *Earle*, and *Tracy* 293, Colorado, Hb. U.S. 76097; fig. *l* (×0.67), from type sheet of *T. rupestre*, *Macoun*, British Columbia, Hb. Can. 15111.

PLATE XXXII

Taraxacum ceratophorum

FIG. *a* (×0.47),—Type material of *T. mutilum*, *Glenn*, Alaska, Hb. U.S. 376755; fig. *b* (×0.36), from type sheet of *T. lacerum*, *Dawson*, northern British-Columbia boundary, Hb. Can. 15119; fig. *c* (×0.44), type plant of *T. leiospermum*; *Osterhout* 2645, Colorado, Hb. N.Y.; fig. *d* (×0.47), authentic material of *T. Chamissonis*, Cole, St. Paul Isl., Hb. U.S. 376691; fig. *e* (×0.37), from one of type sheets of *T. dumetorum*, *Greene*, Wyoming, Hb. Green 48431.

PLATE XXXIII

Taraxacum eriophorum

FIG. *a* (×0.64).—From type sheet of *T. eriophorum*, *Mrs. Fitch*, Montana, Hb. Mont. Agric. Exper. Station; fig. *b* (×0.52) from one of type sheets of *T. angustifolium*, *Greene*, Wyoming, Hb. Greene 48451; fig. *c* (×0.59), from type sheet of *T. ovinum*, *Macoun*, Alberta, Hb. Can. 11711; fig. *d* (×0.47), from type sheet of *T. ammophilum*, *Nelson*, Wyoming, Hb. Greene 48427.

GROUPING AND MUTATION IN BOTRYCHIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 273

(WITH ELEVEN FIGURES)

CHARLES J. CHAMBERLAIN

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GROUPING AND MUTATION IN BOTRYCHIUM

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Charles J. Chamberlain

(WITH ELEVEN FIGURES)

Ever since the appearance of Chrysler's[1] paper, claiming that the fertile spike of *Botrychium* represents a pair of fused pinnae of the vegetative leaf, I have been interested to note the peculiarities in the spore-bearing portions, upon which he relies for a part of his evidence. Two fertile spikes in the position of the lower pair of leaflets, spikes in which the two component leaflets are incompletely fused, sporangia on the second pair of leaflets in addition to the sporangia of the fertile spike, and occasional sporangia on vegetative leaves were found during vacation field studies of *Botrychium obliquum* and its varieties. Chrysler's claim needs no support, his evidence both from field study and anatomy being so convincing that, for years, we have treated these forms as a mere family, Ophioglossaceae, under the Filicales.

However, the field studies, carried on for several years during September vacations in Ohio, at Oberlin, Sullivan, Cleveland, and Birmingham, together with a few observations at Osborn, Indiana, and at Fort Sheridan, Illinois, impressed upon me that one scarcely ever finds isolated plants of *Botrychium*. They are almost invariably grouped; even when there seems to be an isolated plant, others can usually be found in the immediate vicinity.

In *Ophioglossum* there is abundant vegetative propagation by branching of the rhizome, so that not only are the plants grouped, but the plants of a group are more or less connected. In striking contrast, *Botrychium* shows no vegetative propagation. The rhizome scarcely ever branches and, when it does, the branch is not likely to become separated and form a new plant. Every plant comes from a prothallium. Consequently the distribution is

[1] Chrysler, M. A., The nature of the fertile spike in Ophioglossaceae. Ann. Botany 24: 1–18. 1910.

entirely by spores; and since the prothallia are of the subterranean tuberous type, with an endophytic fungus, the prothallia develop only when the conditions for this rather unusual mode of development are present.

How far the spores might be carried is problematical. The grouping of plants indicates that most of the spores are not carried far, but when a plant is once established it becomes the center of a group.

At first I was interested only in the fact of grouping and in the size of the groups of *Botrychium obliquum* and *B. virginianum*. It was noted immediately that the groups of *B. virginianum* contained many more plants than those of *B. obliquum*, and that the groups were more closely associated. In counting plants and making plots, one soon learns to find specimens, especially the smaller ones, which easily escape notice, and the number of plants in a group is likely to be surprisingly larger than the average botanist would have guessed from a cursory examination.

The most closely associated groups, with the largest number of plants in a group, were found at the borders of rather open woods. Plants in the deeper woods, although likely to be large and vigorous, are not abundant.

During the Septembers of the past four years the grouping was observed, and a searching for prothallia developed some facility in recognizing young plants. In 1918 the plants of many groups were counted, especially at Sullivan, where *Botrychium* is exceptionally abundant; and in 1919 plots were made, showing not only the number and position of plants in a group, but also the relation of the groups to each other.

Botrychium virginianum is more abundant than *B. obliquum*, even when the two species are growing together under the same conditions. On the eastern border of a densely wooded tract at Sullivan, Ohio, where *B. virginianum* is more abundant than I have ever seen it in any other locality, prothallia were collected and observations were made for several years. The border of the woods is roughly marked by a rail fence, with but few trees on the eastern side and some trees removed on the western side, so that the woods end in what farmers call a "clearing." The plants and

prothallia are most abundant in the clearing, within 25 m. of the fence, becoming more and more scattered as the woods become denser, while at a distance of 200 m. west of the fence scarcely any plants are found. In this place plants are most abundant on little elevations caused by uprooted trees. When a large tree is blown down, the roots tear up a considerable quantity of soil, so that when the tree decays and disappears there remains a mound with a depression on one side of it. These little mounds of clay soil, scantily covered by moldy humus, seem to be exceptionally favorable places for the germination of spores and the growth of plants.

A few years ago, before any plots were made, the abundance of plants in this locality suggested counting the number on definite areas. These areas do not correspond exactly to the groups which were plotted later, because only the denser centers of the groups were considered, the more scattered plants at the borders being omitted. Some of the highest countings of plants on given areas are worth recording. On areas of 1 sq. m. there were 15, 20, 29, 30, 31, 42, 66, and 106 plants. In the last case the plants were very closely crowded, one cluster of 5 plants occupying a space only 3 cm. sq. On areas of 2 sq. m., there were counted 27, 43, 70, and 103 plants; on 4 sq. m., 112 plants; on 2 dm. sq., 7, 10, and 16 plants; and on 2.3 dm. sq., 8 and 21 plants.

In this clearing the groups were rather closely associated, being separated from each other by distances of 1–10 m., with only here and there a plant between. Many of these plants were small, some of them sporelings still attached to prothallia; but in any place where *Botrychium* is abundant there will be a goodly number of large plants. In such places white patches of the fungus can be seen by turning over the leaf mold.

Aside from noting the grouping and counting the number of plants in a group, little was done with *B. virginianum*. The same must be said of *B. simplex*, which was discovered accidentally at Osborn, Indiana, during a search for *Ophioglossum*. A group of a dozen specimens of this little *Botrychium* was found on an area of about 1 sq. m. So far as we know, this species has not been reported for the Chicago region.

The principal interest centered in *B. obliquum* and *B. dissectum*, which is often regarded as a variety of *B. obliquum*. There are other forms which taxonomists describe as varieties of *B. obliquum* and which may be as distinct and may have as definite a relation to the parent form as we believe *B. dissectum* has to *B. obliquum* but we did not make any study of these forms, and in making plots and in counting we recognized only *B. dissectum*, and put all the rest—the varieties *oneidense*, *tenuifolium*, and *elongatum*—under the general name *B. obliquum*. Besides these varieties, which can often be identified with a manual, there are fluctuating variations, so that one who is not a professional taxonomist is tempted to call the whole assemblage *B. obliquum* and let the name cover *B. obliquum* and its derivatives.

B. obliquum does not occur in such large numbers as *B. virginianum*, the plants of a group being more scattered, with seldom more than a dozen plants on 1 sq. m. This difference in numbers and the difference in grouping is indicated in fig. 1. This plot represents an area of 33 by 40 m. The dots and the crosses of the diagram are all of one size, but the plants varied from sporelings still attached to prothallia up to large specimens. Where plants are so numerous, as indicated in the denser groups, not more than a quarter of them have fruiting spikes.

Why plants of *B. virginianum* should be so much more numerous than those of *B. obliquum*, when they are growing in the same situation, particularly when growing on the same spot, as shown in the lower right hand group in fig. 1, is not obvious. A million spores would be a very conservative estimate for the output of an average plant of either species, and in the largest plants the output probably reaches five or six million spores; but a comparison of the number of plants and the number of spores would indicate that far less than one spore in a million produces a plant which can be seen above ground. One might guess that spores which do not sift down immediately to a safe depth die very soon from exposure or only a little later from the winter's cold; but we have noticed that in the tropical rainy forests of southern Mexico, where *Botrychium* is abundant and where there would seem to be no danger from dryness or cold, prothallia are as difficult to find as in the United

States. It may be possible that differences in the sculpturing on the spore coats may facilitate or impede the penetration of the

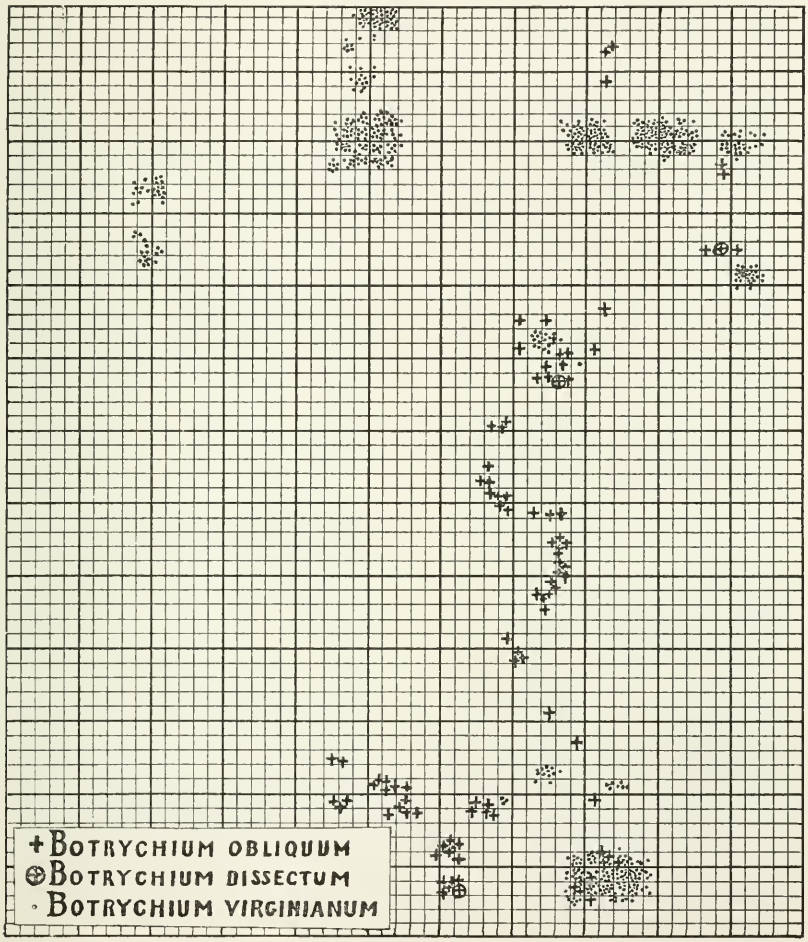


FIG. 1.—Plot 33×40 m. at Sullivan, Ohio, showing *Botrychium virginianum*, *B. obliquum*, and *B. dissectum;* each dot represents a plant of *B. virginianum;* each cross, a plant of *B. obliquum;* and each cross in a circle, a plant of *B. dissectum.*

spores to a favorable depth, or may favor or impede the absorption of water, and thus account for the larger number of plants of *B. virginianum.*

The principal study of *B. obliquum* was made at Oberlin, Ohio, in the cemetery, a part of which is sparsely covered by the original timber, while the rest is still more sparsely dotted with *Juniperus*, *Pinus*, *Thuja*, and *Cupressus*. Of the 24 groups which were counted

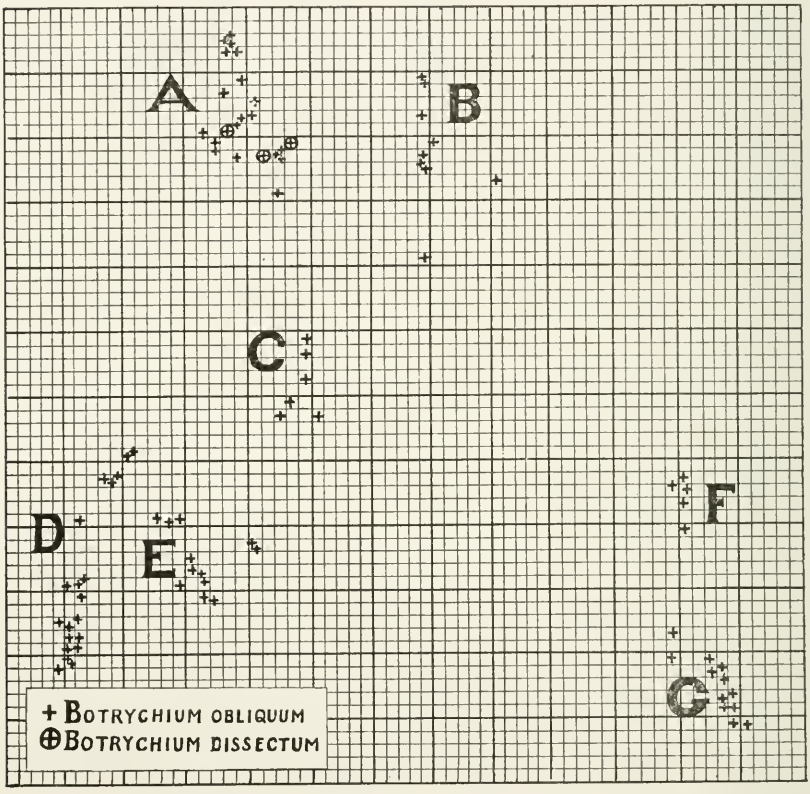


FIG. 2.—Plot about 40×43 m. at Oberlin, Ohio: distances between individual plants of a group approximately correct, but distances between groups *A* and *C*, *C* and *D*, *C* and *F*, and *F* and *G* about twice as great as indicated; there is no *B. virginianum* in this vicinity.

and plotted, 17 were at this place, 4 at Sullivan, 2 at Cleveland, and 1 at Pittsfield. A sample of the plotting at Oberlin is given in fig. 2.

It was from such detailed field studies as those shown in figs. 1 and 2 that we reached the conclusion that *B. dissectum* is a

mutant from *B. obliquum*. In ordinary cases such a conclusion would be tested by sowing the spores and growing the plants; but, so far as we are aware, no one has ever succeeded in raising prothallia of any species of *Botrychium* from the spore. Even if someone should find out how to grow prothallia and sporelings, it would

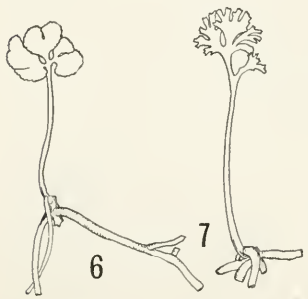
FIGS. 3–5.—Two vigorous plants of *B. obliquum*; plant of *B. dissectum* with typical leaf and roots, but with unusually good sporangia: small plant of *B. obliquum*.

take a long time to secure any results. How long the spore may rest before germinating is problematical; but even after it germinates it is probably a year or more before the prothallium reaches the fertilization stage. In adult plants the leaf is in its fourth year when it appears above ground. Consequently at least five years, and more probably six or eight years, would elapse between the

sowing of the spores and the appearance above ground of the first tiny leaf of the sporeling. We have seen one sporeling with a fertile spike bearing a few sporangia while still attached to the prothallium, but it is probable that ten or twelve years usually elapse between the germination of the spore and the development of a plant up to the spore-bearing stage. During the past twenty years we have made so many unsuccessful attempts to germinate *Botrychium* that we did not even try to test our theory by this method, but collected some circumstantial evidence which supports our conclusion that *B. dissectum* is a mutant from *B. obliquum*.

Before presenting the evidence it is worth while to call attention to the distinguishing characters of the two forms.

B. obliquum and its varieties have oblique leaflets with margins ranging from nearly entire to quite sharply serrate, sometimes doubly serrate, while *B. dissectum* has a leaflet, still oblique in topography, but so dissected that the specific name is very appropriate (figs. 3–5). This difference in leaves is recognizable even in the sporeling (figs. 6 and 7). The leaves of sporelings are simpler in outline than those of larger plants, but the general character of the margins is characteristic from the first, so that there is no danger of confusing the forms.



FIGS. 6, 7.—Sporeling of *B. obliquum;* natural size; sporeling of *B. dissectum;* natural size.

In *B. obliquum* and its varieties there is considerable variation in the shape of the leaflet and in the character of the margin; but, so far as the margin is concerned, the differences are confined to a greater or lesser degree of serration. The deepest serration of *B. obliquum* would not be mistaken for the deeply cut margins of *B. dissectum*. In *B. dissectum* there is also some variation in the margins, but the dissected character is always evident, the differences being in the extent of the dissection (fig. 8).

We are familiar, of course, with the great variations in the leaflets of cultivated ferns, where a single leaf may have leaflets with a nearly entire margin, leaflets deeply cut, and still others so

deeply cut that the bipinnate condition is reached. We do not regard *Botrychium* as a similar case, but believe that the differences in the margins of *B. obliquum* and *B. dissectum* are more like the differences in the margins of the leaflets of *Bowenia spectabilis* and *B. serrulata*, and like the differences in the leaf margins of *Dioon edule* and *D. spinulosum*. In these four cycads, the margins are so constant that they are reliable diagnostic characters.

The short subterranean stem, with the long-stalked leaf and spore-bearing portion with a still longer stalk, is similar in *B. obliquum* and *B. dissectum*.

The roots of *B. dissectum* are wrinkled and fleshy, like those of *B. obliquum*, and not at all like the slender roots figured in BRITTON and BROWN'S *Illustrated Flora*.

In general, *B. obliquum* is a larger plant than *B. dissectum*. At Oberlin the largest plant of *B. obliquum* measured 35 cm. in height, with a leaf 20 cm. in width, and the spore-bearing part of the fertile spike 15 cm. long. While this is not quite up to the limit in size recorded for the species, it is very large, and most individuals are much smaller. One plant of *B. dissectum* measured 28 cm. in height, but this is exceptional. The usual size of *B. dissectum* is about two-thirds that of *B. obliquum*.

FIG. 8.—Leaflets from three plants of *B. dissectum;* natural size.

The most suggestive difference between *B. obliquum* and *B. dissectum* is seen in the fertile spike. The sporangia of *B. dissectum* sometimes look uniform and perfect, but somewhat smaller than those of *B. obliquum*. The difference in size, where the sporangia seem to be perfect, may be seen by comparing *A* and *D* of fig. 9.

However, most specimens show a considerable proportion of abortive sporangia which, even without sectioning, may be distinguished by their smaller size (fig. 9, *B* and *C*). The figure of *B. dissectum* in BRITTON and BROWN'S *Illustrated Flora* is evidently

drawn from a specimen with sporangia like those in fig. 9, *B* and *C*, and in this respect is characteristic.

Sections of sporangia, like those shown in fig. 9, *B* and *C*, show that the smaller sporangia contain no spores at all (fig. 10). In most of these cases the sporangium wall is from 4 to 6 cells thick, with the inner layer not differentiated into a definite tapetum, and the outer lacking the anticlinal thickenings so characteristic of sporangia which produce even imperfect spores (fig. 11). In extreme cases the sporangium is a mere mass of parenchyma cells; in others, a narrow streak of mucilage indicates that sporogenous tissue had begun to form; in still others, like the one shown in

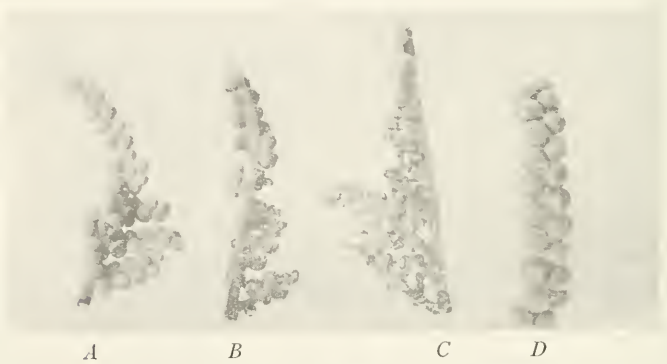


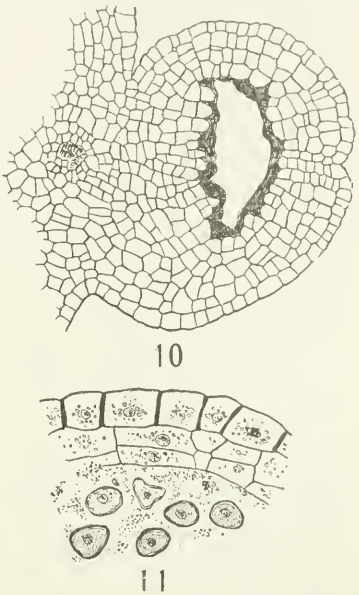
FIG. 9.—*A*, *B*, *C*, *B. dissectum*; *D*, *B. obliquum*; ×2

fig. 10, a considerable mass of sporogenous tissue has been formed; and in a few cases it could be seen that the mucilaginous mass consisted partly of imperfect, disorganizing spores.

In the apparently perfect sporangia of *B. dissectum* many of the spores are somewhat smaller than the average size for *B. obliquum*, and there are many spores which look as if they might be abortive. Fig. 11 shows six spores still floating in the tapetal plasmodium. The two spores at the upper left, one of them triangular in outline, are doubtless abortive; of the other four, only the one at the lower left has the full diameter of a normal spore of *B. obliquum*. The epidermal layer has anticlinal thickenings, as in normal sporangia, which dehisce and shed their spores.

It would be interesting to compare the reduction divisions of the two species, but the problem of getting material of *B. dissectum* makes such a comparison difficult, if not impossible. Even with good preparations of critical stages, the interpretation might be uncertain, for, judging by a few figures in *B. obliquum*, the $2x$ number is well over 100, and the chromosomes are tangled and hard to count.

Such evidence as we have would indicate that *B. dissectum* is at least partially, and probably entirely, sterile. Unfortunately the natural test which would prove or disprove this theory—germinating the spores—cannot be applied until someone learns how to make these baffling spores germinate. If the spores of *B. dissectum* germinate, we do not see why this species should not occur in groups, like *B. virginianum*, *B. obliquum*, *B. simplex*, and probably the other species.



FIGS. 10, 11.—Abortive sporangium of *B. dissectum*, with many-layered wall and mass of mucilage lining cavity from which sporogenous tissue has been resorbed; ×160: portion of sporangium of *B. dissectum*, showing spores of different sizes; triangular spore doubtless abortive; ×350.

In our opinion, the explanation of the occurrence and behavior of *B. dissectum* is that the species is a sterile mutant from *B. obliquum*. The principal facts supporting this theory are that *B. dissectum*, so far as I have observed in a five years' study, never occurs except in association with *B. obliquum*, and that there is no evidence that it reproduces itself.

It might be objected that mutants do not occur so frequently as *B. dissectum* would indicate, and it must be admitted that the total number of plants in our plots is not as large as one might wish in making ratios. The total number of plants in the twenty-four plots was 482 of *B. obliquum* and 19 of *B. dissectum*, a ratio

of 251:. The ratio in the Oberlin group was 20:1; in the Sullivan group, about 48:1; and in the Cleveland group, 40:1.

However, in making any objections to the theory on the ground that *B. dissectum* occurs too frequently to be a mutant, it must be remembered that mutation in plants has been studied almost exclusively in angiosperms, which are heterosporous and which have comparatively low chromosome numbers. We are assuming that mutations occur in the mitotic mechanism, probably during the reduction divisions, so that the mutant, which one recognizes in the sporeling stage, is merely the result of a preceding phenomenon.

At first thought, someone might suggest that *B. dissectum* is a hybrid. What species could cross to give such characters as we find in *B. dissectum?* The mere question seems a sufficient answer, especially since *B. dissectum* is found when no other species except *B. obliquum* occurs in the vicinity. When we remember that the prothallia are of the tuberous, subterranean, saprophyte type, and not closely associated, the possibility of crossing seems very remote.

We believe the evidence is sufficient to raise a strong presumption that *B. dissectum* is a sterile mutant from *B. obliquum.*

University of Chicago

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ORIGIN OF MECHANISM OF HEREDITY

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Merle C. Coulter

The gross features of the mechanism of heredity have become features of general knowledge. The majority of biologists think of heredity in terms of determiners located upon the chromosomes. There are certain critical details of the mechanism, however, which still remain profoundly obscure. Little is known of the exact nature of the determiners themselves. The orderliness in the behavior of the determiners, that is, how they are "released" to express themselves only at the appropriate moments in the life history of the organism, seems not to have been clearly visualized. Finally, the possible origin of this mechanism of heredity is seldom even discussed. The present paper suggests, although only in a very brief and general way, certain answers to these questions.

It seems safe to assume that the most primitive organism lacked not only an organized nucleus, but even the components of a nucleus. A consideration of the activities of such an organism will reveal a suggestion as to the origin of the hereditary mechanism, provided, of course, that the assumptions are sound. The metabolism of this primitive organism, in certain fundamental features, will be similar to that of all organisms. Raw materials will be taken in and transformed to provide building materials and energy. If the raw materials be pure and the machinery of the protoplast perfect, this transformation will be complete, so that all the raw materials taken in will be transformed and used. Actually, however, the raw materials provided are never quite pure, and the machinery of the protoplast, although infinitely more efficient than any man-made machine, must be subject to certain flaws and frictions of its own. The transformation and use of materials, therefore, will not be complete; certain waste materials and by-products will remain. We are not concerned with the waste material; it is the fate of the by-products which is significant.

Certain of the by-products may be insignificant in their influence upon the protoplast. Others will undoubtedly be toxic in their effect, as many investigations upon auto-intoxication have gone to show. Primitive excretory systems, developed primarily for the disposal of waste materials, may remove a considerable part of these by-products. Thorough cleansing of the protoplast by this method, however, is impossible. Inevitably by-products will accumulate with the age of the organism. In fact age itself, in other than the purely chronological sense, is probably accounted for by this very accumulation of by-products. The toxic influence of these by-products will interfere with the efficient working of the machinery of the protoplast, and metabolism will be slowed down; hence "old age." Rejuvenescence occurs with cell division, because at cell division the protoplast is cleansed of many of these toxic by-products. This cleansing probably involves both physical and chemical forces. Physical reorganization at cell division will explain the exposure of these by-products; chemical oxidation will account for their removal (as toxins).

Again, were the machinery of this cleansing process a perfect one, rejuvenescence would be complete. Actually, however, the cleansing of the protoplast at cell division is not (or is not always) absolutely thorough. A few of the by-products pass over to the daughter protoplasts. The daughters, therefore, start life with a few by-products which the mother did not possess at the beginning of her life. Since these by-products are toxic and impair or retard metabolism, it is evident that the daughters are, at birth, slightly "older" than was the mother.

A series of repetitions of this performance through successive generations will have a cumulative effect. As a consequence, not only does the individual grow old through ontogeny, but, in a very real sense, the whole race is gradually aging through phylogeny. Evidence is not lacking that the higher organisms, cell for cell, have a lower rate of metabolism than do the more primitive ones. This is a statement of the quantitative effect of these by-products. It is their qualitative effect, however, that casts light upon the origin of the hereditary mechanism.

The by-products which originally accumulated in the protoplast were of various types. Some were very toxic, and these, if they were not immediately eliminated, resulted in the death of the organism. Others were less toxic, relatively more harmonious with the protoplast itself. These last, since they were not immediately fatal, stimulated an adaptive response on the part of the protoplast.

As to the general nature of this adaptive response, an important assumption must be made. Recent researches upon mammals have revealed in these organisms the power to develop antitoxins. The presence of a small quantity of toxin stimulates the organism to an adaptive response, the development of an antitoxin specific for the toxin present. This power is probably one of the fundamental characteristics of all protoplasm, being present even in the most primitive organisms.

Certain by-products in the primitive organism, only slightly toxic in their effect, stimulated it to produce an antibody. The protoplast is doubtless a colloidal system, and we may consider antibodies in the following light. The antibody counteracted the influence of the toxic by-product by insulating it from contact with the protoplast. Antibodies were probably developed most successfully for those by-products which were the least toxic in their effect. These by-products then became insulated by the antibodies. This insulation was significant not only in cutting off the influence of the by-product upon the protoplast, but in another respect also. At cell division this by-product, even though exposed, is not oxidized because of the protection afforded by the antibody which insulates it. It is probably this mechanism, for the most part, which accounts for the fact that some of the by-products are passed on to the daughter protoplasts, as mentioned before. These by-products are the primitive bearers of hereditary characters. The program carried out by the primitive hereditary mechanism is as follows.

The life of the primitive organism, like that of all organisms, involves a series of reactions. Early in the life of the organism there is present a certain reaction system, characterized by certain physical and chemical conditions. For a time the reaction system

as a whole maintains a sort of equilibrium, in which only reactions of a certain type are possible. This may be referred to as the *A* equilibrium. Inevitably, through the accumulation of certain materials, the *A* equilibrium will be upset. After a period of readjustment, the *B* equilibrium will succeed; this will be followed by the *C* equilibrium, and so on. The total number of distinct equilibria in the life program of any organism probably is in rough proportion to phylogenetic age.

Taking as an example the *X* equilibrium, certain questions may be considered. What is it that accounts for the existence of the *X* equilibrium? It is the inevitable result of reactions which took place during the *W* equilibrium. The *W* equilibrium may be similarly accounted for by the previous existence of the *V* equilibrium. The program is an inevitable one, and will be followed during each generation.

Under conditions imposed by the *X* equilibrium, only reactions of a certain type are possible, and these may be referred to as the *x* reactions. A number of *x* reactions are possible, x_1, x_2, x_3, etc. Chance conditions (environment, directly or indirectly) will determine which of these will take place. Whichever takes place, there will result a by-product, and this by-product will be of the *x* type. Even more specific than this, the x_3 reaction will result in and be characterized by the x_3 by-product. As the existence of the *X* equilibrium was inevitable, there will inevitably be laid down one of the *x* type of by-products. Since, however, it was chance which specifically selected the x_3 reaction, this same chance is indirectly responsible for the by-product x_3, rather than x_1, x_2, or any of the other possibilities.

The by-product x_3 will exist in an active state and exert some influence upon the protoplast so long as the *X* equilibrium continues. The eventual disappearance of the *X* equilibrium will be accompanied and characterized by the insulation of by-product x_3 by means of a specific antibody which has been developed by the protoplast. The *Y* equilibrium will follow; and, just as the *X* equilibrium was characterized by the free active existence of by-product x_3, one of the characteristics of the *Y* equilibrium will be the existence of x_3 in an insulated inactive condition. The by-

product x_3, insulated by its antibody, will pass on at cell division to the daughter protoplast. Early in the life of the daughter there must exist an A equilibrium. The inevitable program will then be followed, until finally the X equilibrium is reached. A very critical assumption is made at this point. The insulation of x_3 by its antibody is a phenomenon of colloidal chemistry. Similar colloidal reactions are known to be reversible. The formation of the antibody for x_3 took place at the inception of the Y equilibrium, which was characterized by the effective insulation of x_3. The X equilibrium, however, which now recurs during the following generation, is conducive to the free and active existence of any by-product of the x type. When the X equilibrium is reached in the life of the daughter protoplast, therefore, a dissolution of the antibody will occur and x_3 will be released.

With the X equilibrium now in existence, it is certain that reactions of the x type will take place. Which one of the possible x reactions occurred was in the first generation a matter of chance. In the present instance, however, the presence of by-product x_3 will exert a determining influence. The result, eliminating external stimuli of an unusual intensity, will be that the x_3 reaction and the x_3 by-product again stimulate the protoplast in a characteristic manner, developing in the daughter the same characteristic that was present at a similar stage in the life of the mother.

This theory accounts for the origin of the hereditary mechanism in terms of by-products and antibodies which insulate them. These various antibodies must form an important constituent of "modern" chromosomes, but there must also be present some more stable and homogeneous framework.

The release of the determiners (by-products) at the appropriate moment is referred to phenomena of colloidal chemistry. It is an open question whether this release is reflected by visible changes in the chromosomes. If so, a given locus on a chromosome should be seen in a loose or "open" condition only during a brief phase of the life history. No doubt this point would be hopeless to ascertain in any very accurate way.

As for the determiners themselves, these are visualized as by-products of metabolism, chemically active substances. The

reaction system (for example, X equilibrium), which arises as the result of an inevitable sequence of events, determines what general type of reaction shall take place at a given phase in the life history. The by-product (for example, x_3) merely decides which of a number of possible reactions within this general type shall be the one chosen.

The origin of a given by-product was accounted for by chance environmental conditions. The environment referred to may well have been the external environment in the case of the simpler organisms, but must be the internal environment in the more complex. This seems also to provide sufficient basis to explain the small degree of inheritance of acquired characters that has been said to take place.

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AFTER-RIPENING AND GERMINATION OF JUNIPERUS SEEDS

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AFTER-RIPENING AND GERMINATION OF JUNIPERUS SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 275

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(WITH ONE FIGURE)

Some seeds fail to germinate in compensating percentages or even at all when placed under ordinary germination conditions. Because of inquiries directed to this laboratory from various growers concerning the best methods of handling juniper seeds, there was conducted a careful study of after-ripening, germination, and seedling development, as well as some of the chemical and physiological changes involved in these processes. Strict quarantine laws, recently put into effect, will mean that many species of decorative plants that were formerly grown from seeds in foreign countries and brought to America as plants, must now be grown from seeds by American nurserymen. This will doubtless promote study of the germinative behavior of many refractory seeds in the future.

Literature

Wild plants of the temperate zone produce seeds that usually have a rest period, which varies as to length and cause with the different species and kinds of seeds. This dormancy is found to be characteristic of the seeds of 75 per cent of the wild and the cultivated plants studied by HOWARD (18). Although the rest period of most seeds is only a few months, it may be years, as in the case of some Conifers (21) and of *Euphorbia Cyparissias* (19). CROCKER (5) states that delayed germination is due to one or more of the following conditions: (1) rudimentary embryo, (2) dormant embryo, (3) coats inhibiting embryo expansion, (4) coats inhibiting gas exchange, (5) coats inhibiting water absorption, (6) a combination of two or more of these, and (7) secondary dormancy. Up to date seeds have been studied that represent each of these different types of dormancy.

As it has been impossible to dispense with this rest period in all cases, many substances have been used to reduce dormancy and force seeds to germinate. Concentrated sulphuric acid has been used by HILTNER and KINZEL (17), ROSTRUP (32), and others with positive results. Among the salts ROSE (31) noted that the sulphates and nitrates were the better forcing agents. Hydrogen peroxide and increased oxygen pressure forced the germination of *Xanthium* seeds (5). Wounding and treatment with ether stimulated the germination process (3). Light has been found to force or to inhibit germination depending on the seed (12, 20). The New York Experiment Station (24) and many others have shown that desiccation improves the germinating power of corn. The hot bath has been used with success on some seeds (4). Alternating temperatures have been used to force grass seeds in the Seed-testing Laboratories of the Bureau of Plant Industry. With these much has been claimed for freezing and thawing as a forcing agent (29). LAKON (21), however, found that the germination of *Pinus Peuce*, *P. Cembra*, *P. Strobus*, and *P. silvestris* could not be accelerated by treatment with dry heat, warm bath, file injury, ether, chloroform, salt solutions, concentrated sulphuric, or dilute acids.

Seeds with dormant embryos must go through a series of changes (after-ripening) before germination can occur (5). The after-ripening of hawthorn seeds proceeds fastest at 5–6° C. according to DAVIS and ROSE (8). An idea of this after-ripening process may be gained by following the results of LAKON on a protein and ECKERSON (10) on a fatty seed. LAKON (22), in studying the changes that precede germination of *Fraxinus excelsior*, found very little increase in water absorption. From the tenth day on, starch accumulated in the embryo cells, with a corresponding disappearance of protein from the endosperm cells. In place of the disappearing protein a turbid emulsion formed, which later was digested. At no time did starch appear in these endosperm cells. The embryo doubled its length during this process of "Vorkeimung." ECKERSON (10) studied the changes occurring in the hawthorn seed during after-ripening, and reported an increasing acidity and water absorbing power of the dormant organ. The catalase, peroxidase, and oxidase activity increased as after-ripening and

germination proceeded. Germination was accompanied by a decrease of stored fats and an increase of sugar. Although the details varied somewhat, both seeds passed through a period of preparation for germination.

Material and preliminary study

The *Juniperus* plants are erect or prostrate dioecious Cupresseae distributed over the Northern Hemisphere. They are used in landscape decoration, serving as hedges and screens up to 30 ft. high. In early spring the flowers appear in the leaf axils, forming many carpel whorls, of which only the upper one develops. This whorl bears 3 ovules, which grow together and form a spherical fruit, which requires two years to ripen, and contains 1–3 seeds.

TABLE I

MATERIAL SECURED

Species	Lot	Date	Place
J. virginiana L.	1	November 11, 1918	West Newberry, Massachusetts
J. c. depressa Pursh.	2	January 1, 1919	Boxford, Massachusetts
J. communis L.	3	January 1, 1919	Vermont
J. prostrata Pres.	4	January 1, 1919	Vermont
J. virginiana L.	5	January 1, 1919	Vermont
J. communis L.	6	April 19, 1919	Near Chicago, Illinois
J. virginiana L.	7	April 19, 1919	Near Chicago, Illinois
J. communis L.	8	September 19, 1919	Near Chicago, Illinois
J. virginana L.	9	September 19, 1919	Near Chicago, Illinois

Juniperus seeds were gathered in the fruit condition, and those used in these experiments were collected as stated in table I.

The seeds freed from the fruit vary with the species as to color, shape, size, and quality. Those of *J. virginiana* are light brown smooth, brittle, 3–4 mm. long, and when air-dry weigh about 0.009 gm. each. Seeds of *J. c. depressa*, *J. communis*, and *J. prostrata* are much alike. These seeds are dark amber, rough, 4–6 mm. long, narrower and less brittle than those of *J. virginiana*. Some of the *J. virginiana* material proved to be badly worm eaten, while the other lots were quite free from worms. Seeds collected in Vermont were generally good. Table II gives the percentage of bad seeds due to worms and lack of development.

Fig. 1 shows the structure of the seed of *J. virginiana*, with its many membranes and protective layers. In strong contrast with the hard brown coat are the clear white endosperm and embryo. The hard coat consists of three layers: the outer fleshy (*a*), the stony (*b*), and the heavy inner fleshy (*c*). In the outer fleshy layer are found pectic substances and methyl pentosans. The stony layer is lignified and contains other substances, as calcium, pectates, and pentoses. The inner fleshy layer is well developed and consists of suberin with some little cellulose. Of the endosperm, embryo, etc., one distinguishes the nucellus (*d*), the mass of distorted tissue (*e*), the hypocotyl cap (*f*), the megaspore membrane (*j*), the endosperm wall (*k*), the endosperm (*g*), and the embryo (*h*).

TABLE II

PERCENTAGE OF IMPERFECT SEEDS IN LOTS 1, 3, 4, AND 5

Species	Lot	No. examined	Percentage imperfect
J. virginiana	1	100	59
J. virginiana	1	50	63
J. virginiana	1	100	61
J. virginiana	1	2000	60
J. communis	3	1000	26
J. prostrata	4	1000	20
J. virginiana	5	2000	22.5

The nucellus is constructed of long narrow cells which give tests for cellulose and pectic acid. The mass of tissue (*e*) protecting the hypocotyl consists of cellulose, pectic substances, and some other groups of substances such as fats and gums. Between this mass and the hypocotyl is a cap of very fine and firm cells (*i*), which are made up of cellulose and hemicellulose. The megaspore membrane (*j*) is very thin and stains with ruthenium red. Examination shows that the outer wall of the outer layer of endosperm cells has been developed into a suberin wall (*k*). This wall is insoluble in concentrated H_2SO_4, 50 per cent chromic acid, and gives the phellic acid reaction. The endosperm cell walls are rather thick and made up of cellulose. Cell walls of the embryo are thin and consist of pectose and cellulose.

The storage substances of the resting seed are mentioned here, but they are later taken up in detail with the changes accompanying germination. Tannin is generally distributed throughout the coat. Some is stored in the nucellus, endosperm wall, and hypocotyl cap, but none was found in the endosperm or embryo. The embryo and endosperm are stored with an abundance of protein and fat, and also a trace of glucose. No starch was found in either endosperm or embryo. Histidine, tyrosine, and arginine are found in both endosperm and embryo. There is also a trace of leucine and probably cystine.

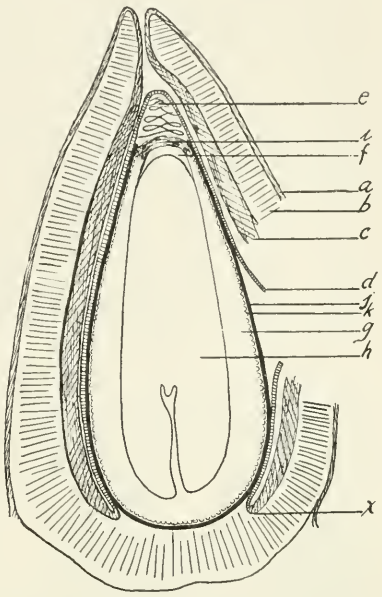

FIG. 1.—Longitudinal section of seed of *Juniperus virginiana* with part of nucellus and integument removed from one side: *a*, outer fleshy; *b*, stony; *c*, inner fleshy; *d*, nucellus; *e*, distorted tissue; *f*, hypocotyl cap; *i*, protective cap; *j*, megaspore membrane; *k*, endosperm wall; *g*, endosperm; *h*, embryo.

Catalase activity of the embryo and endosperm is low, while that of the coat is negligible. The seed shows peroxidase activity, with a mere trace of oxidase activity.

The resting seed embryo has a P_H value of about 8, while the endosperm has a P_H value of about 5. Thus the embryo is basic, while the endosperm is acid, a condition opposite to that usually found in seeds which are ready for germination.

Treatment of material

After collection the larger part of the fruit or berry was removed from the seed by running the berries through a coffee mill so wide open as not to injure the seed. Next the seeds were sifted and the milling and sifting repeated. The seed material was then rubbed between two sieves in the presence of an abundance of water. In this way all the berry and excess tissues which prevent

sterilization were easily and quickly removed. The bad seeds were floated off with water, and the good seeds rinsed and permitted to dry before sterilization.

After some testing, a 5 per cent solution of formalin acting for 2.5 minutes was selected as the best sterilizing agent for juniper seeds. It was found that formalin did not readily penetrate the coat, reduce the catalase activity, or hinder germination. The permeability of the coat was studied as follows. Seeds were submerged in different solutions for a definite time, removed, washed in distilled water, the coats removed, the seeds sectioned with a freezing microtome, and the sections tested for the respective solutions. Table III shows that the coat was very permeable to water, bases, and salts, but not permeable to stains and acids.

TABLE III

PERMEABILITY OF COATS TO WATER, STAINS, ACIDS, BASES, FORMALIN, AND SALTS

Substance	Permeability	Substance	Permeability
Eosin (dilute).....	Impermeable	Water.....	Very permeable
Eosin (strong).....	Impermeable	C_2H_5OH.....	Very permeable
Neutral red (dilute)..	Impermeable	NaOH.....	Very permeable
Neutral red (strong).	Impermeable	KOH.....	Permeable
Formalin.....	Slowly permeable	NH_4OH.....	Permeable
HCl N/100.....	Very impermeable	$AgNO_3$.....	Very permeable
H_2SO_4 N/100	Very impermeable	$HgCl_2$.....	Very permeable

That the salts ($AgNO_3$ and $HgCl_2$) penetrated the coats is shown by the catalase activity of the seeds with coats removed (table IV). These seeds, after being sterilized, washed, and incubated at 9° C. for 48 hours, had coats removed, and were ground for catalase activity determinations. It was further shown that $AgNO_3$ penetrated the coats by the fact that seeds so treated were killed. Seeds sterilized in formalin germinated, and therefore most of the seeds used in these experiments were sterilized 2.5 minutes in 5 per cent formalin. In this connection it should be noted that SCHROEDER (33) and GROVES (14) found that the coat of the wheat seed was practically impermeable to $AgNO_3$, and that this solution was a good sterilizing agent for wheat. This shows how the permeability of seed coats may vary with different seeds.

For some experiments the seeds were freed from the probable inhibiting influence of the hard coats by one of the three following treatments: (1) dry seeds were put into concentrated H_2SO_4 and the rate of penetration followed by testing with congo red; it required 24 hours to entirely carbonize the coat; the carbonized coats were rubbed off with filter paper and the seeds rinsed in a suspension of $CaCO_3$ and distilled water; (2) seed coats were also removed with seed nippers; (3) in other experiments only the end of the coat was cut away. The sterile seeds were put into sterile wide mouthed bottles, Petri dishes, or flasks for germination. Those cultures

TABLE IV

EFFECT OF STERILIZING AGENTS ON CATALASE ACTIVITY OF SEEDS, TREATED 2 MINUTES (40 SEED COATS REMOVED)

STERILIZING AGENTS	OXYGEN IN CC. LIBERATED DURING		
	1 min.	5 min.	10 min.
Water (check)	9.0	20.0	24.3
Water (check)	8.9	19.0	24.0
Formalin 5 per cent	8.5	21.0	24.8
Formalin 5 per cent	8.8	21.2	24.4
$AgNO_3$ 2 per cent	3.0	6.0	8.0
$AgNO_3$ 2 per cent	3.1	6.4	8.1
$HgCl_2$ 1 per cent	4.0	11.2	13.0
$HgCl_2$ 1 per cent	4.1	10.0	11.8

which required good ventilation were protected against infection by a system of tubes plugged with cotton. The seeds were left on the moist walls of the containers or on moist filter paper, depending upon the conditions of the experiments. In the determination of the effect of solutions as forcing agents, no foreign absorbing material was allowed in the flasks with the seeds. In all other cases, except where mentioned, the seeds were placed on moist filter paper.

Forcing agents

The change of the catalase activity and the ability of the seeds to germinate were used as standards to determine whether or not the substance or treatment under examination was a forcing agent for the juniper seed.

METHODS.—As it has been shown that catalase activity increases with after-ripening of the dormant-embryo seeds (**6, 10**), catalase activity was chosen as the first standard. Germination, the production of independent seedlings, was selected as the final standard. Great care was found necessary in the preparation of material and the manipulation of the catalase apparatus. As the berry has a high catalase activity, every trace of fruit was removed before grinding. The grinding was carried out under similar conditions, and with a definite amount of water and no. 2 sand per unit weight of seed material. The dioxogen was neutralized with N/10 NaOH at the time of using. All determinations were made with the bath at 25° C., and the drive wheel of the apparatus regulated to make 30 revolutions per 10 seconds. No explanation is needed for germination as a standard.

FORCING AGENTS.—Among the common forcing agents tried on the juniper seeds were high temperatures, alternating temperatures, removal of coats, hydrogen peroxide, dilute acids, carbon dioxide, light, soil, mercuric chloride, ether, and oxygen. The first seven of these had very little effect on the catalase activity and did not force germination. While the treatments with mercuric chloride, ether, and oxygen did not force germination, each had its influence upon catalase activity.

CROCKER and HARRINGTON, in an unpublished work at the Seed-testing Laboratories of the Bureau of Plant Industry, have found that $HgCl_2$ was a good forcing agent for Johnson grass. Juniper seeds were sterilized and put into flasks containing the following concentrations of $HgCl_2$. After 24 hours the excess of liquid was poured off. The results are given in table V. These data, as well as those obtained by grinding the seeds for catalase activity in the same concentrations, show that $HgCl_2$ reduced catalase activity in the higher concentrations. None of the seeds treated with $HgCl_2$ germinated.

In studying the effect of ether, seeds were sterilized, put into Petri dishes without covers, and exposed to air containing various amounts of ether by scaling in 9 liter cans. After a certain exposure the seeds were removed, aired, and placed to germinate. Table VI gives the catalase activity for seeds that were exposed to ether

6 days and then left in the germinator 95 days at 25° C. Seeds similarly treated, except that they were exposed only 2 days, gave less marked catalase activity. The point to be noted in table VI is the increased activity which was given by seeds treated with the larger amounts of ether. Of added interest is the fact that there

TABLE V

CATALASE ACTIVITY OF SEEDS TREATED 95 DAYS WITH $HgCl_2$ SOLUTIONS AND PERCENTAGE OF GERMINATION AFTER 6 MONTHS

CONCENTRATION OF $HgCl_2$ USED	OXYGEN IN CC. LIBERATED DURING		
	1 min.	5 min.	10 min.
N/800	0.5	2.3	2.9
N/1600	1.6	3.0	3.4
N/3200	1.3	3.1	3.9
N/6400	2.5	3.5	4.0
N/12800	3.0	4.0	4.6
N/25600	3.0	4.5	5.0
N/51200	3.2	5.0	5.9
N/102400	2.9	5.5	6.2
Water (check)	2.8	5.5	6.0

TABLE VI

EFFECT OF ETHER ON CATALASE ACTIVITY OF JUNIPER SEEDS KEPT AT 25° C.

AMOUNT OF ETHER PER 9 LITERS OF AIR	OXYGEN IN CC. LIBERATED DURING		
	1 min.	5 min.	10 min.
1.4 cc	2.9	6.0	6.1
2.8 cc	3.5	7.8	8.4
5.6 cc	4.8	10.0	10.9
8.4 cc	5.0	10.5	11.0
16.8 cc	5.6	11.0	11.5
Without ether (check)	2.8	5.5	6.0

was no germination. It is suggested, therefore, that one may have an enormous increase of catalase activity without a corresponding after-ripening of the dormant embryo.

To study the effect of oxygen, seeds were sterilized and exposed to air containing the following percentages of oxygen. Table VII gives the catalase activity for seeds at the end of 45 days. With the higher percentage of oxygen there was an increase of catalase

activity. At the forty-fifth day the remaining seeds were exposed to atmospheric air. Table VIII gives the catalase activity for the same lot of seeds at the end of 95 days, 50 days after replacing the oxygen by air. The point of interest here is the fall in catalase

TABLE VII

EFFECT OF OXYGEN ON CATALASE ACTIVITY OF SEEDS STORED 45 DAYS AT 25° C.

PERCENTAGE OF OXYGEN	LOT	OXYGEN IN CC. LIBERATED DURING		
		1 min.	5 min.	10 min.
30	1	2.5	4.4	4.8
55	2	3.4	5.3	5.6
80	3	3.7	6.2	6.6
100	4	4.8	7.5	8.8
Air (check)		3.8	6.2	6.9

TABLE VIII

REDUCED CATALASE ACTIVITY IN OXYGEN TREATED SEEDS WITH DECREASE IN PERCENTAGE OF OXYGEN

LOT	OXYGEN IN CC. LIBERATED DURING		
	1 min.	5 min.	10 min.
1	2.8	5.0	6.0
2	2.9	5.1	6.1
3	2.7	5.0	5.8
4	2.8	5.0	5.6
Air (check)	2.8	5.5	6.0

activity, at the ninety-fifth day, for the seeds that were exposed to 100 per cent O_2 during the first 45 days. None of these seeds germinated.

TEMPERATURE.—No other condition affected the development of the juniper seeds to the extent that temperature did. Both alternating and constant temperatures ranging from 15–30° C. were found to reduce the catalase activity and inhibit germination. Seeds exposed to winter weather (in soil and on moist filter paper) gave about 1 per cent germination. Those subjected to a temperature of 10–12° C. in running water showed a steady increase of catalase activity up to the time of germination. Between the

fourth and sixth month the germination reached 10 per cent, a very marked increase over that obtained at the higher temperatures. When the temperature of the water rose much above 12° C. germination ceased. These results show that the increased germination was not due to the removal of inhibiting substances from the coat, but to the effect of the low constant temperature.

Although many observers (**11, 20, 29, 30**) have reported a forcing action for freezing and freezing with thawing, these results show that when freezing really occurs it is very injurious. On March 14, 1919, 1000 air-dry seeds and 1000 moist seeds were placed at a constant temperature of −23° C.; and 1000 air-dry and 1000 moist seeds were subjected to an alternation of temperature between −23° and 10° C. The latter seeds were left at each temperature for one week. After 45, 95, and 150 days of exposure samples were removed for study. The catalase activity of these seeds for 45 and 95 days is given in table IX. The catalase activity of seeds stored dry at −23° C. equaled that of untreated seeds, while that of seeds stored wet at −23° C. and wet or dry at the alternating temperature showed a marked decrease. There was no change in the oxidase or peroxidase activity. The seeds stored dry at −23° C. showed no increase of H^+ ion or titratable acid over that of the untreated seed. All other seeds showed a slight increase of sugar content and of H^+ ion concentration; also a 40 per cent gain of titratable acid. Both embryo and endosperm of these seeds, stored at the alternating temperatures, had the same H^+ ion concentration. The fats in these seeds were very soluble, not characteristic, and diffused throughout the endosperm and embryo. This general diffusion of the fats and the equal H^+ ion concentration for the embryo and endosperm indicate that the membranes had become more permeable by freezing (**16**). On staining these seeds with methylene blue they appeared to be dead. Samples of all seeds were put under favorable conditions for after-ripening and germination, but only the seeds that were stored dry at −23° C. after-ripened and germinated. These results prove that these low temperatures are very injurious unless the seeds are dry. It is probable that seeds stored at this low constant temperature and protected from moisture would retain their viability many years.

The alternation of temperature between −23° and 10° C. kills moist *Juniperus* seeds very soon, and in no sense can be looked upon as a forcing agent.

One lot of moist seeds was subjected to a temperature of −5° C., and a second lot to an alternating temperature of −5 and 5° C. The latter was exposed to each temperature for one week

TABLE IX

EFFECT OF TEMPERATURE ON CATALASE ACTIVITY OF JUNIPER SEEDS (NO. 10)

Material and treatment			O_2 in cc. liberated during			Total loss or gain over that of dry seeds (cc.)
Condition	Weight in gm.	Temperature °C.	1 min.	5 min.	10 min.	
	Stored 45 days					
Dry	0.025	.....	2.6	5.4	5.8	.....
Dry	0.025	−23	2.5	5.4	5.7	−0.1
Wet	0.026	−23	2.4	5.0	5.5	−0.3
Dry	0.023	−23 and +10*	2.2	4.9	5.4	−0.4
Wet	0.026	−23 and +10*	1.6	3.2	3.6	−2.2
Wet	0.025	− 5	3.0	5.1	5.8	0.0
Wet	0.025	0	3.0	6.0	6.5	+0.7
Wet	0.026	− 5 and + 5*	3.5	6.8	7.0	+1.2
Wet	0.026	+ 5	3.6	7.5	9.1	+3.3
Wet	0.026	+10	3.5	6.6	7.0	+1.2
Wet	0.026	+25	3.0	6.2	6.9	+1.1
	Stored 95 days					
Dry	0.023	−23	2.3	5.5	5.8	0.0
Wet	0.023	−23	2.4	5.1	5.7	−0.1
Dry	0.026	−23 and +10*	2.0	4.2	5.0	−0.8
Wet	0.025	−23 and +10*	1.0	2.8	3.1	−2.7
Wet	0.025	− 5	3.0	6.4	6.5	+0.7
Wet	0.025	0	3.4	7.0	8.0	+2.2
Wet	0.026	− 5 and + 5*	3.8	8.0	9.0	+3.2
Wet	0.026	+ 5	5.0	9.1	12.0	+6.2
Wet	0.026	+10	3.9	8.2	9.2	+3.4
Wet	0.026	+25	2.8	5.5	6.0	+0.2

*Weekly alternated between the two temperatures.

at a time. Table IX shows the catalase activity of these seeds for 45 and 95 days exposure. The lot stored at −5° C. showed scarcely any increase of catalase activity, while the lot exposed to the alternating temperature was more active. Both lots appeared morphologically and physiologically in good condition. There was a slight accumulation of sugar in all seeds. The first showed

no germination, but some of the latter germinated after about 6 months. While exposure to $-5°$ C. was not sufficient to injure the ungerminated seeds, it proved fatal to the germinated seeds. This is due to the fact that when the coat splits open the endosperm and embryo just doubles its water content and thereby dilutes the cell sap to a degree which permits ice crystals to form. Seeds at this period and later periods of development were killed by exposure to $-5°$ C. for seven days or less. The after-ripening and gain in catalase activity was a little more than enough to account for the sum of the effect at 5° C. These results show that the alternation of temperature between $-5°$ and 5° C. had slight forcing action. This forcing action is equal to that obtained by keeping seeds in running water at 10° C. It is also evident that seeds ready to germinate should not be subjected to $-5°$ C.

The early changes taking place in seeds put to germinate at $0 \pm 1°$ C. were similar to those at 5° C. except for being retarded. At this temperature the increase in catalase activity was very much retarded, although it was over 3 times that gained by seeds stored at $-5°$ C. per unit time. These seeds were studied as to storage material, H^+ ion concentration, and permeability, and found in good condition. The *Juniperus* seeds not only afterripened but germinated at $0 \pm 1°$ C., even though it required 5 or 6 months.

Moist seeds were placed at 5° C. for germination. At this temperature the catalase activity increased most rapidly. The physiological changes occurring in the seed at 5° C. were most rapid, and will be discussed in detail under changes preparatory to germination. This constant temperature of 5° C. also gave rise to by far the largest percentage of germination, and the most vigorous seedlings.

It is evident from these germination experiments that: (1) temperatures above 10° C. and below 0° C. are not favorable for afterripening and germination; (2) no one of the forcing agents as used was of value in germination; (3) the inclosing structures do not inhibit germination; (4) but the inhibiting conditions are to be found in the endosperm and embryo. These facts indicate that the juniper seed has a dormant embryo that must go through

a series of fundamental changes before germination. Of the many points of attack that are suggested by these experiments two were chosen: (1) changes preparatory to germination, and (2) means of shortening the after-ripening period.

Changes preparatory to germination

These are the changes that occur in the seeds stored at 5° C. which prepare them for germination. As the embryo of the dry seed is morphologically complete, increases very little in size, and shows only the transformation of cell contents, these processes could be spoken of as "foregermination"; but as this term has not been used in this country these processes will be referred to as after-ripening. The first point studied was the imbibition of water.

TABLE X

SHOWING PERCENTAGE ABSORPTION OF WATER

(SEEDS DRIED AT 105° C. FOR MOISTURE DETERMINATION)

Material	Percentage water	Weight	Weight after submergence for hours indicated									Percentage water at maximum imbibition
			2	4	8	16	24	72	96	120	360	
Entire seed	7.00	2.0018	2.16	2.37	2.40	2.41	2.42	2.42	2.43	2.42	2.39	23.22
Endosperm and embryo coats off during imbibition	7.19	0.290	.....	.....	.....	.....	0.37	0.38	.....	0.37	.....	28.94
Endosperm and embryo coats on during imbibition	7.19	0.291	.....	.....	.....	.....	.....	.....	0.375	.....	.....	24.84

Table X shows that the seeds decreased slightly in weight after a few days, even when submerged in water. In examining the tables given by LAKON for the water absorption of seeds of *Pinus*, it was noted that he incidentally obtained similar results. To follow this more closely, seeds with coats on were placed on moist filter paper at 5° C., and at times samples were selected, coats removed, and the percentage of water in the seed, exclusive of coat, determined. Table XI gives these results and the percentage of water in the seedlings as well. It should be noted that the water content of the seed decreased gradually until germination, when there appeared a very marked increase up to the time of the developed seedling. This percentage of water seems to be related to the change in the water absorbing power of seed contents, and

not to changes in the permeability of the coat, as later experiments show.

Table XII gives the changes of H^+ ion concentration as P_H values for the endosperm and different parts of the embryo during storage at 5° C. The outer cells of the embryo and its hypocotyl were the first parts to show an increased H^+ ion concentration.

TABLE XI

PERCENTAGE OF WATER IN SEEDS TAKEN FROM COATS AFTER DIFFERENT PERIODS OF EXPOSURE TO 5° C.

Seeds	Percentage water
Dry	7.19
After 5 days at 5° C	24.84
After 15 days at 5° C	23.34
After 30 days at 5° C	23.09
After 60 days at 5° C	23.00
After 90 days at 5° C	23.21
After 100 days at 5° C. (coat splitting open)	52.64
After 130 days at 5° C. (seedlings 25 mm. long)	88.38

TABLE XII

H^+ ION CONCENTRATION OF SEEDS DURING AFTER-RIPENING*

Condition	Part of seed	P_H
Dry	Endosperm	4.4–6.0
Dry	Embryo	8.4–8.8
After 30 days at 5° C	Endosperm	4.6–5.2
After 30 days at 5° C	Embryo	6.8–7.6
After 60 days at 5° C	Endosperm	4.4–6.0
After 60 days at 5° C	Embryo	6.8–7.6
After 90 days at 5° C	Embryo hypocotyl	6.0–6.8
After 90 days at 5° C	Endosperm	4.4–5.2
After 90 days at 5° C	Embryo outer cells and hypocotyl	4.4–5.2
After 90 days at 5° C	Embryo inner cells	4.6–6.0

* These determinations were made with the Clark and Lubs indicators.

The embryo showed a marked increase of H^+ ion concentration during after-ripening, while the endosperm with P_H value of 4.4 (concentration of H^+ ions $\times N = 0.72 \times 10^{-4}$), being already acid, showed very little change. This may indicate that the embryo is the principal seat of dormancy. Table XIII gives the increase of titratable acid in the endosperm and embryo during after-ripening. The increased acid was determined by titrating with N/50 NaOH,

using phenolphthalein as the indicator. To show that this increased acid content is real the calculated dry weight of the seed material used is given.

The fat of the dry seeds is stored as very large globules, contrary to the statements of CZAPEK, which divide and become continually smaller as after-ripening goes on. Just preceding germination these fat globules, in the active growing cells, become reduced to microscopic size, although CZAPEK states that the microscopically divided fat of dry seeds collects into globules with early

TABLE XIII

INCREASE OF TITRATABLE ACID IN ENDOSPERM AND EMBRYO

Condition	No.	Dry weight in gm.	N/50 NaOH in cc.	Increased acid per unit volume of water
Dry	80	0.165	0.56	.....
Dry	160	0.348	1.25	.....
Dry	80	0.160	0.53	.....
Dry	80	0.164	0.55	0.0254
After 15 days at 5° C.	80	0.163	0.57	0.0989
After 30 days at 5° C.	80	0.172	0.58	.....
After 30 days at 5° C.	80	0.172	0.60	0.0866
After 60 days at 5° C.	80	0.169	0.70	.....
After 60 days at 5° C.	80	0.167	0.70	0.0685
After 95 days at 5° C.	80	0.157	0.85	.....
After 95 days at 5° C.	80	0.163	0.90	0.0564
Open seeds	80	0.189	1.50	0.1426
Hypocotyl 2 mm	80	0.195	2.60	0.1615
Seedlings	80	0.210	8.00	.....
Seedlings	80	0.204	8.00	0.1865

growth (7). This dispersion of the fatty material brings clearly into play surface tension, adsorption power, and many other forces resulting from the great increase of specific surface. Such a dispersion could lead to a more rapid digestion of the fats, thus materially aiding the transformation of fats to carbohydrates and the accumulation of energy for germination. The importance of making the fats more capable of transformation to carbohydrates should not be overlooked. It is also probable that this dispersion reaches a degree of division where it could aid in the translocation of fats as such. Thus highly dispersed fatty material would be carried through the cell walls at points of protoplasmic connection.

Early during the process of after-ripening there was a slight decrease in the fat content of the endosperm cells surrounding the embryo. The most rapid disappearance of fat occurred in the hypocotyl end of the endosperm at approximately the ninety-fifth day. This rapid decrease of fat was accompanied by an increase in the sugar content of the adjoining hypocotyl cells. This was the first noticeable increase of sugar during after-ripening. At this time the coat splits open, probably partly due to the increased osmotic pressure of the newly synthesized sugar. With these changes the first detectable starch was found. It increased very rapidly in these cells, until they seemed to be completely packed. Traces of starch appeared in the cotyledons and they soon became green, a point to be taken up later. Thus during the preparation for germination the stored fat was transformed into carbohydrates. Not all the fat is changed directly into carbohydrates. Under certain conditions it seems to be changed into forms more capable of translocation and used to synthesize other compounds, or even stored again. It seems that a large part of the food material of these seeds during after-ripening, germination, and the development of the seedling is translocated in this form.

Amino acids appear in both ungerminated (dry) and germinated seeds. Table XIV gives the amino acids found in these feeds, as well as a rough estimate of their quantities. The histidine in the endosperm was used up completely during the after-ripening.

Table XV gives the changes occurring in the proteins of *Juniperus* seeds during germination as indicated by color reaction. These results show that soluble proteins increased during after-ripening. It was also shown that the proteins were hydrolyzed during after-ripening by the determination of amino nitrogen and the formal titration. Table XVI gives the results of the VAN SLYKE determination for amino acids. This table shows that the 5 minute reaction period was too short, which indicates the presence of amino acids with other than α-amino groups. The arginine found would account for the increase under 30 minutes reaction. These figures prove that there was a marked hydrolysis of the proteins during after-ripening, as well as during germination and the development of

seedlings. As this protein digestion goes on, the number of free amino groups increases because of the splitting amino-carboxyl linkings. When hydrogen of the free amino group is replaced by

TABLE XIV

TESTS FOR AMINO ACIDS IN JUNIPER SEEDS

AMINO ACIDS	CRYSTALLIZATION	COLOR REACTIONS	AMOUNT OF AMINO ACIDS IN			
			Dry seeds		After-ripened seeds	
			Endosperm	Embryo	Endosperm	Embryo
Histidine...	+	Ehrlich's diazo	+++	+++	+	++
Tyrosine....	+	Ehrlich's diazo	+	+	++	++
Tyrosine....	+	Millons	+	+	++	++
Tyrosine....	+	Xanthroproteic	+	+	++	++
Cystine....	+	Sulphur reduction	+	+	+	+
Leucine....	+	.....	+	+	++	++
Arginine....	+	.....	+	+	++	++

TABLE XV

CHANGES IN STORED PROTEIN FOOD DURING GERMINATION

REACTIONS	DRY SEEDS			AFTER-RIPENED SEEDS		
	Endosperm	Embryo	Hypocotyl	Endosperm	Embryo	Hypocotyl
Biuret.....	++	+++	+++	+	+	++
Millons.....	+++	+++	+++	++	++	+
Xanthroproteic.....	+++	++	++	+++	++	+
Berlin blue.....	+	?	?	+	++	+++

TABLE XVI

INCREASE OF AMINO NITROGEN DURING AFTER-RIPENING AND GERMINATION

Condition of seed material	Time of reaction (min.)	N (cc.)	Temperature	Pressure (mm.)	Nitrogen obtained (mg.)	Amino acid as percentage of dry weight
Dry or resting.....	5	0.25	23.2	753.2	0.138	0.035
Coats bursted after 100 days at 5° C.....	5	0.50	24.7	750.8	0.274	0.270
Hypocotyl 3 mm. long or after 105 days at 5° C.....	5	0.59	24.5	751.0	0.324	0.275
Developed seedling or after 130 days at 5° C.....	5	1.41	24.5	751.3	0.775	0.921
Dry or resting.....	30	0.60	23.2	753.2	0.332	0.036
Coats bursted after 100 days at 5° C.....	30	0.84	24.2	750.7	0.462	0.279
Hypocotyl 3 mm. long or after 105 days at 5° C.....	30	1.17	24.5	750.7	0.642	0.280
Developed seedling or after 130 days at 5° C.....	30	1.97	25.2	750.7	1.078	0.935

methylene, the basicity becomes reduced; and the substituted acid can then be titrated with sodium hydrate as a measure of protein hydrolysis. Titrations made on a second lot of seeds according to the SORENSEN method gave results similar to the VAN SLYKE determinations.

The growth in these seeds occurring before germination is very meager. There is no morphological change in endosperm or embryo, although the latter increased slightly in length. After the appearance of sugar the hypocotyl exerts a forward pressure, separating the sides of the swelling cap which forces the coat open. At this moment the cap is under so much pressure that it is distorted, and a sharp angle is formed between its end and sides. The growth following this stage will be discussed later.

TABLE XVII

RESPIRATION OF SEEDS AT DIFFERENT PERIODS OF DEVELOPMENT AT 25° C. (7 CC. VOLUME)

Condition of seeds	No.	Green weight	Days	Percentage CO_2	Percentage CO_2+O_2	Percentage O_2 used	$\frac{CO_2}{O_2}$	Mgm. CO_2 per hour per gm.	Mgm. O_2 per hour per gm.
Dry.....	500	1.250	5	1.18	20.82	1.57	0.76	0.00098	0.0011
After 5 days at 5° C.	50	0.125	1	3.15	20.39	3.77	0.84	0.1311	0.1347
After 30 days at 5° C.	10	0.030	3	3.78	20.82	3.98	0.94	0.218	0.1976
After 60 days at 5° C.	10	0.027	3	3.80	20.70	3.90	0.97	0.2352	0.2151
After 90 days at 5° C.	10	0.028	3	3.80	20.68	3.90	0.97	0.2354	0.2075
After 100 days at 5° C.	10	0.028	3	4.10	20.00	6.00	0.68	0.2486	0.3192
After 130 days at 5° C.	10	0.099	1	9.30	20.56	9.74	0.95	0.4890	0.4398

Table XVII gives the results of the respiration experiments obtained by the use of the Bonnier and Mangin apparatus. There was a great increase in the respiratory intensity during the first 5 days and after the seeds split open. These are the periods when the seed increased in water content. There was a very slow increase in the respiratory intensity during after-ripening, even though the water content decreased. The respiration quotient increased very slightly during after-ripening, but decreased to a minimum at germination. Not only does this low respiratory quotient of 0.68 indicate the time of intense fat metabolism, but at this particular period it was found that the fats were being transformed into carbohydrates. It would be interesting to know this quotient at 5° C., as it would probably be much lower. After germination

the seedlings gradually attained the ratio 1:1. This rise in the respiratory quotient was probably due to the oxidation of carbohydrates and the more intense respiration of the seedlings.

Table XVIII gives the results of intramolecular respiration. The method used was that of NICOLAS (26). The point to be noted here is the low 1/N ratio (the intramolecular or anaerobic respiration divided by the normal respiration) for the seedlings.

Peroxidase was more generally present than oxidase. Quantitative oxidase activity determinations were made with the Bunzel apparatus. These results showed that there was no appreciable increase of oxidase activity until after germination.

TABLE XVIII

INTRAMOLECULAR RESPIRATION OF JUNIPER SEEDS, NO. 10, AT 25° C. (7 CC. VOLUME

Condition of seeds	Weight	Days	Percentage CO_2	1/N ratio
After 30 days at 5° C.	0.030	3	1.70	0.44
After 90 days at 5° C.	0.028	3	1.67	0.43
After 100 days at 5° C.	0.028	3	1.66	0.40
After 130 days at 5° C.	0.099	1	0.95	0.10

The results of catalase determinations are given in table IX, which gives the average of a great number of experiments. It was found that (1) when seeds were placed under ordinary germination conditions at 5° C. the increase of catalase activity gave a measure of the after-ripening; (2) the gain in catalase activity above that of air-dry seeds was greatest at 5° C. in a germinator; (3) the gain at the other temperatures was slow at best; and (4) seeds soon lose their catalase activity when in a germinator at temperatures above 25° C. The precautions used in the catalase determinations have been stated.

CROCKER (6) speaks of the rise in vigor of seeds, as shown by their resistance to fungal attack, during after-ripening. The juniper seed is protected against fungi before germination by the heavy lignin coat. It was found that juniper seeds which had not been after-ripened soon succumbed to fungal growths with the removal of the coats. After-ripened juniper seeds, however, when freed from the coats, withstood dense fungal growths.

Many such experiments indicate that the vigor and resistance of the seed to fungi increased greatly during the after-ripening process. These results prove that the juniper seed has a dormant embryo that goes through certain definite and well defined fundamental chemical and physical changes before germination can occur. Some changes occur also in the endosperm.

SHORTENING AFTER-RIPENING PERIOD AT 5° C.—The after-ripening period was shortened considerably by the constant temperature of 5° C., as has been shown, but attempts to shorten further this after-ripening period at 5° C. seemed to meet with difficulties. GUPPY'S (15) method of forcing seeds to germinate by placing the soft pre-resting seeds (caught before going into the rest period) at 20° C. was tested. None of these seeds germinated, and it is evident that the juniper seed must pass through a more or less definite rest and after-ripening period. This period was not shortened by the removal of the seed coats. ECKERSON (10) states that dilute acids greatly shorten the after-ripening period of the hawthorn. Dilutions of HCl between N/100 and N/3200 had no effect upon the juniper seed. Neither sugar, enzyme, nor vitamine solutions shortened this period. Hydrogen peroxide gave no results. In the treatment with different percentages of oxygen, it was found that the catalase activity increased slightly with increased oxygen pressure, and that the germination was retarded two months. Seeds were treated with different percentages of ether ranging from 0.002 to 6.000. As long as these seeds were under the influence of ether they showed a decrease in catalase activity proportional to the percentage of ether used. After atmospheric conditions were restored, all seeds recovered their catalase activity, but the after-ripening period was lengthened from 1 to 3 months depending on the low and higher percentages of ether. If the ether acted by decreasing the permeability, then it was evidently reversible, contrary to the work of OSTERHOUT (27). It is more probable, however, that the ether acted as a narcotic agent. This is also shown by the behavior of the seed. Carbon dioxide was used in concentrations ranging from 0.5 to 100 per cent with a six day exposure. The higher percentages increased the catalase activity and shortened slightly the after-ripening period. The action here

was probably due to increased acidulation in the presence of an abundance of CO_2 and H_2O which could favor the digestion of fats and germination (25). Desiccation and moistening again of seeds at about the forty-fifth day after being placed in the germinator shortens the after-ripening period from 5 to 10 days. This may be due to one of the following causes: (1) earlier after-ripening of the coats, as they are found to split off more readily when desiccated, (2) upsetting of the chemical equilibrium by the great extraction of water, or (3) the increase of H^+ ion concentrations.

Discussion

CATALASE.—The catalase activity, as has been noted by previous investigators (1, 6), was found to bear some relation to respiration. Increased catalase activity accompanied the intense respiration of *Juniperus* seeds stored in high percentages of oxygen, as decreased

TABLE XIX

INCREASED CATALASE ACTIVITY WITH DEVELOPMENT (FIGURED PER UNIT DRY WEIGHT)

CONDITION OF SEEDS	OXYGEN IN CC. LIBERATED DURING		
	1 min.	5 min.	10 min.
Air dry	2.5	5.4	5.8
After 45 days at 5° C.	3.6	7.5	9.1
After 95 days at 5° C.	5.0	9.1	12.0
After 100 days at 5° C. (coats split)	5.3	12.2	14.4
After 130 days at 5° C. (seedlings)	10.5	23.1	28.5

catalase activity accompanied the low respiration of seeds stored in low percentages of oxgyen. With the intense respiration at high temperatures there was an increased catalase activity, even though the seeds did not after-ripen or germinate. The highest catalase activity and the most intense respiration per unit of dry weight was found in the seedling stage (cf. tables XIX and XVII). The desiccation of seeds to a slight extent, which makes for a rapid absorption of oxygen through the coat, increased the catalase activity. Desiccation to the extent of retarding respiration reduced the catalase activity. Table XX shows these results. Both the respiration and the catalase activity of seeds were reduced at will

by submerging them in water. Although increased catalase activity generally accompanied intense respiration, this relationship did not always hold, for when seeds were submerged a long time the catalase activity slowly increased, but there was no increase of respiration intensity. An examination of tables IX and XVII will show that the catalase gain was proportionally very much larger than the respiration gain during after-ripening. It will also be noted that the catalase gain was greatest at 5° C., where the respiration was low. It is evident, therefore, that there may be increased catalase activity without an increase of respiration.

TABLE XX

CATALASE ACTIVITY OF AFTER-RIPENED AND DESICCATED SEEDS, NO. 30 (CALCULATED DRY WEIGHT 0.0696)

Treatment	O_2 cc. liberated after 10 min.
Complete imbibition	33
Slight dessication	38
Strong dessication	32
Second imbibition	36

RATE AND PERCENTAGE OF GERMINATION.—Juniper seeds germinate most readily at the low temperature of 5° C. These seeds germinate, although very slowly, at 0±1° C. They also germinate at 10° C. Seeds after-ripened at 5° C. and then placed at 10° C. germinated slower than those left at 5° C. After-ripened seeds were thrown into a state of secondary dormancy by exposure to temperatures above 12° C. Their catalase activity gradually decreased and germination ceased. After being thrown into secondary dormancy, several weeks at 5° C. were required to after-ripen the seeds again. The seeds which sank in water gave between 75 and 80 per cent germination at 5° C.

GROWTH OF SEEDLING.—Table XXI gives the rate and extent of growth for seedlings exposed to the light or the dark at different temperatures. All seeds were germinated at 5° C. and then transferred to the different temperatures. The length of the extending hypocotyl at the time of transfer was 0–1 mm. The seedlings grew the longest and fastest at 25° C. At 30° C. they never attained

a normal length, while at 0± 1° C. there was a slow but definite growth. It is important to note that 15° C. seedlings developed first and appeared the most healthy and sturdy. These seedlings

TABLE XXI

EFFECT OF LIGHT AND TEMPERATURE ON RATE AND EXTENT OF GROWTH*

TEMPERATURE	LIGHT	LENGTH OF HYPOCOTYL IN MM. FROM TIME OF TRANSFER					
		3 days	7 days	11 days	13 days	18 days	26 days
30° C...	Dark	1	5	7	12	18	20
25° C...	Dark	10	35	40	55	60	Seedling
15° C...	Dark	4	18	29	Seedling	.....	.....
10° C...	Dark	3	10	30	35	Seedling	.....
10° C...	Light	3	11	29	30	Seedling	.....
5° C...	Dark	2.5	4	12	15	26	35
0± 1° C...	Dark	0	1	2	3	4	5
− 5° C...	Dark	0	Killed	0	0	0	0

*Average of 50 trials.

at 15° C. also showed the earliest and greatest development o chlorophyll. Light did not seem to affect unusually the extent or rate of growth.

PIGMENTS.—Carbohydrates and temperature may condition chlorophyll development. The seedling was found to develop chlorophyll in total darkness. Thus the cotyledons become green long before they break out of the coat. Chlorophyll appeared first in the cotyledons and accompanied the formation of starch. This points to the conclusion that soluble carbohydrates are necessary for the formation of chlorophyll, the view advanced by PALLADIN (28). Table XXII gives the results of experiments planned to determine the effect of light and temperature on greening. This shows that light affects in no way the rate or apparent depth of greening. It also shows that at 30° C. and at 0± 1° C. chlorophyll did not develop. As the plastids were found to be in good condition, it was thought probable that a lack of building material was inhibiting chlorophyll development. Glucose cultures were made, therefore, but the seedlings again failed to develop chlorophyll. This indicates that a certain temperature is necessary for chlorophyll development, regardless of carbohydrate supply,

the maximum, optimum, and minimum temperatures for chlorophyll formation in the seedlings being represented by temperatures somewhat below 30, 15, and somewhat above 0° C.

Seedlings grown at 0± 1° C. developed anthocyanin, while those grown at 30° C. developed xanthophyll. When cultures at 0± 1° C. were supplied with glucose they developed more anthocyanin. The seedlings grown at 30° C. were made to develop anthocyanin by the addition of glucose. From the foregoing it appears that the seedlings form various pigments according to their reserve sugar

TABLE XXII

EFFECT OF LIGHT AND TEMPERATURE ON DEVELOPMENT OF CHLOROPHYLL*

TEMPERATURE	LIGHT	ESTIMATED PERCENTAGE OF COLOR AFTER TRANSFER								
		1 day	4 days	6 days	8 days	11 days	13 days	18 days	26 days	50 days
30° C.	Light	0	0	0	0	0	0	0	0	0
25° C.	Light	5	25	50	50	50	65	.....	.....	.....
15° C.	Light	5	25	50	75	75	100	.....	.....	.....
10° C.	Light	5	25	50	75	.....	.....	100	.....	.....
10° C.	Dark	5	25	50	75	.....	.....	100	.....	.....
5° C.	Light	.....	.....	.....	5	.....	.....	50	.....	.....
5° C.	Dark	.....	.....	.....	5	.....	.....	50	.....	.....
0± 1° C.	Light	.....	.....	.....	.....	.....	.....	.....	.....	0
0± 1° C.	Dark	.....	.....	.....	.....	.....	.....	.....	.....	0

*Average of 50 trials.

supply. Seedlings with little sugar tend to develop xanthophyll, those with more sugar chlorophyll, and those with an abundance of sugar anthocyanin.

PRACTICAL APPLICATION.—The foregoing experiments make it possible to devise an outline for the practical production of juniper plants. This should be of interest to growers, since it has furnished a means of increasing many fold the percentage of germination and of developed seedlings. After collection, the seeds are freed from the berries, sorted, and sterilized as has been described. The seeds are then put into Petri dishes or covered flat vessels on filter paper supported by wet cotton. These vessels of seeds are kept at a constant temperature of about 5° C. (41° F.) for after-ripening, which takes about 100 days. This after-ripening period can be shortened 10 days by drying slightly and moistening again the seeds at about the forty-fifth day. When the coats have split

open and the hypocotyls are $\frac{1}{8}$ in. long, the seedlings are transferred to pans or beds of leaf mold and sand kept at 15° C. (60° F.). In no case should ungerminated seeds (seeds that have not split open and developed a short hypocotyl) be transferred from the germinator at 5° C. (41° F.). The germinated seeds, after being transferred to beds or pans, should be protected by glass plates and paper for the first few days.

Although these seeds have been germinating during every month of the year, advantage can be taken of the temperature conditions by placing them in the germinator about January. The importance of this after-ripening and germination at 5° C. cannot be overemphasized.

Summary

1. The germination of non-after-ripened juniper seeds under ordinary conditions is very low, amounting to 1 per cent.

2. These seeds are protected by a semipermeable and thick coat which makes up 75 per cent by weight of the entire seed. Acids enter very slowly, while bases, silver and mercury salts enter rapidly. While the coat serves as a protection against fungal attack and prevents water-imbibed seeds from expanding and rupturing the tissues before after-ripening is accomplished, it takes little or no part in the dormancy or after-ripening of the seed.

3. Food material in the resting seed is stored in the form of ats and proteins, with traces of glucose but no starch. The resting seed endosperm has a P_H value of about 5, while that of the embryo is about 8.

4. Although some forcing agents changed the respiration and catalase activity of seeds, it was not possible to force the germination of non-after-ripening juniper seeds by high temperature, alternating temperature, wounding, warm bath, dry air, removal of coats, treatment with hydrogen peroxide, mercuric chloride, ether, carbon dioxide, oxygen, light, soil, dilute acids, dilute bases, nitrates, suphates, or strong acids.

5. Freezing and thawing as such has no forcing action on the germination of juniper seeds, neither does it hasten after-ripening. Freezing and thawing produces marked chemical changes in this

seed, but these changes, as has been outlined, are quite different from those occurring during after-ripening. Seeds ready to germinate (after the coat is cracked and their water content increased to 52 per cent) are killed by an exposure to $-5°$ C.

6. The juniper seed has a dormant embryo that must after-ripen before germination. After-ripening occurs at temperatures between $0 \pm 1°$ C. and 10° C., although fastest at about 5° C.

7. The changes that accompany after-ripening of the juniper seed at 5° C. were found to be as follows: (1) rather rapid and complete imbibition, followed by a steady slow decrease in water content during after-ripening or until near germination; (2) increased H^+ ion concentration, especially of the embryo; (3) an increment of titratable acid; (4) a steady and enormous increase in the degree of dispersion of the stored fat; (5) decrease in the amount of stored fat and protein, with an increase of sugar content and the first appearance of starch; (6) the translocation of food in the form of fat or fatty acids from endosperm to embryo; (7) a seven-fold increase in the amino acid content, and a complete disappearance of histidine from the endosperm; (8) an increase of soluble proteins with a marked hydrolysis of the stored proteins: (9) slight growth of embryo; (10) very slight increase of the respiration intensity; (11) increased respiratory quotient; (12) decreased intramolecular respiration; (13) a doubling of the catalase activity; and (14) the rise in vigor of seeds as shown by their resistance to fungal attack.

8. In conjunction with after-ripening at 5° C., desiccation seems to be the only promising means of shortening this after-ripening period.

9. The time at which the hypocotyl breaks through the nucellus was fixed as the end of after-ripening and the beginning of germination.

10. Neither the resting nor the after-ripened juniper seeds yield more than about 1 per cent germination at temperatures above 15° C. Seeds after-ripened at 5° C., then placed at 10° C., germinate slower than those left at 5° C. When after-ripened seeds are transferred from 5° C. to temperatures above 15° C. they are thrown into a state of secondary dormancy. Hence these seeds require a low temperature for germination as well as for after-ripening, and therefore no seed should be transferred to

higher temperatures until germination has started. If these seeds are given sufficient time they will germinate, even at $0 \pm 1°$ C.

11. Subsequent to after-ripening and germination at 5° C., the best temperature for seedling development is 15° C.

12. The development of chlorophyll in the juniper seed and seedling was found to be independent of light, but conditioned by the temperature range. Seedlings grown at temperatures of $0 \pm 1°$ C. or 30° C. never developed chlorophyll. Anthocyanin development in seedlings seems to depend upon relative temperature and carbohydrate supply.

13. A more complete chemical analysis of these seeds at different stages of development will be given in a later paper.

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