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THE BOTANICAL GAZETTE

EDITOR
JOHN MERLE COULTER

VOLUME LXIX

JANUARY–JUNE 1920

WITH TWENTY-THREE PLATES AND ONE HUNDRED NINETY-FOUR FIGURES

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ERRATA

Vol. LXVIII

P. 431, table I, column 4, for gm. read cm.

P. 435, table II, column 5, for min. read mm.

Vol. LXIX

P. 27, add description of plate V, as follows:

Enterobryus compressus Thaxter

Fig. 47.—Group of 9 individuals producing cysts, with exception of *a*, young, and *b*, one still bearing normal terminal segment showing attachment to chitinous integument about anus of host

Fig. 48.—Distal end of cyst-forming individual

Fig. 49.—Base of same individual showing attachment and scattered nuclei

Fig. 50.—Terminal portion of individual from which normal terminal segment has separated

Fig. 51.—Similar termination in which cyst formation has commenced, showing nuclei more crowded in region of cyst formation

Fig. 52.—Termination of cyst series

P. 173, line 8 from bottom, for immediately, after adding, read immediately and after adding

P. 179, line 7 from bottom, for monocotyledonous read dicotyledonous

P. 243, fig. 15, for magnification ×1200 read ×600

P. 301, table III, **2.4,** 3.0, 3.85, and 3.9 belong to culm 3 in pot DD; 1.9 and 1.6 belong to culm 4 in same pot

P. 303, table V, line 5 under distance, for increased read same

P. 374, line 3 from bottom, for 0.098*x* read 0.0098*x*

P. 376, last line, for 0.0286*x* read 0.0216*x*

P. 385, line 6 from bottom, after (0.025*x*+1) insert +1.65

P. 396, line 6 from top, for figs. 1 and 11 read figs. 6 and 11

VOLUME LXIX NUMBER 1

THE
BOTANICAL GAZETTE

JANUARY 1920

SECOND NOTE ON CERTAIN PECULIAR FUNGUS-PARASITES OF LIVING INSECTS[1]

ROLAND THAXTER

(WITH PLATES I–V)

Although the examination of mycological novelties possesses a certain fascination, it may have its drawbacks, since in the present, as in numerous other instances that might be mentioned, their interest may be neutralized to a considerable extent by their very novelty, which may be of such a nature as to make it impossible to assign them a satisfactory position among their fellows, or to arrive at any reasonable conclusion as to the true significance of their characteristics. Although from the point of view of the systematic mycologist, and for his greater peace of mind, Nature might well have been better employed than in elaborating organisms which, as far as one can see, are in one way or another interlopers in the scheme of organic life, it seems desirable to assemble them as they appear, since the inevitable accessions to their numbers may ultimately be expected to supply, in a majority of cases, some reasonable explanation of their characteristics, which will make it possible to distribute them satisfactorily in their mycological pigeonholes.

This situation seems to be well illustrated by many of the forms included in these parasites of living insects, which if their isolation were less striking would claim more attention, and have to be put aside until the discovery of similar and related forms

[1] Contribution from the Cryptogamic Laboratories of Harvard University, no. 85.

may serve to make clear their significance. The Laboulbeniales, being a microcosm in themselves, need no apologist; since, despite their unsolved origin, their general position in the fungus series is perfectly clear, except possibly to a few Brefeldians; and, once they have originated, their extraordinary development is quite intelligible. With our present knowledge as a guide, however, the same can hardly be said of the other external fungus-parasites of living insects included in this and in my previous paper[2] on the same subject. Even in the case of genera like *Muiogone* and *Muiaria*, the similarity of which to well known types is manifest, it would be very difficult satisfactorily to explain their manifestly unsuccessful mode of life, the disadvantages of which seem clearly indicated by their rarity, both as regards individuals and species. While such forms may be looked upon rather as outcasts from their proper groups, however, there are others, like *Coreomycetopsis*, the Thaxteriolae, and Enterobryae, which must be regarded as essentially isolated.

This assemblage of species has been obtained from various parts of the world, on insects belonging to numerous different genera of the Coleoptera, Diptera, Orthoptera, and Neuroptera, the most curious forms having been found on Termites, already a classic ground for the parisitologist. Although the first, *Cantharosphaeria*, which is a true ascomycete, may perhaps prove to be, in a sense, saprophytic, with no very definite relation to the vital activities of its host, this can hardly be said of any of the others, the life of which is evidently thus conditioned. *Termitaria*, *Muiogone*, *Muiaria*, and *Aposporella* belong to the Fungi Imperfecti; the first referable in an artificial way to the Leptostromaceae, but quite isolated in its characters; the last, one of the Mucedineae, belonging to a group which includes a number of forms as yet unpublished, having a similar mode of life, and characterized by an absence of differentiated spores, among which the species herewith illustrated is, in some respects, the most striking. *Muiogone* and *Muiaria*, of which species have been previously described, belong to the Dematiae. The position of all the remaining forms, however, is problematical, and, although from its cytological characters

[2] BOT. GAZ. 58:235–253. *pls. 16–19.* 1914.

Enterobryus may be assumed to belong to the Phycomycetes, evident affinities with other members of this class are lacking.

Cantharosphaeria, nov. gen.—Perithecia superficial, scattered, subdimidiate, membranaceous, ostiolate, the ostiole surrounded by a tuft of hairs. Asci 8-spored, aparaphysate; spores hyalodidymous.

Cantharosphaeria chilensis, nov. sp. (figs. 1–5).—Perithecia associated with a rather scanty mycelium of thick-walled, brown, branching hyphae; subhemispherical, blackish brown, slightly roughened, seated on the chitinous integument among the bristles of the host, about 70–80 μ by 40–45 μ; the apical hairs usually closely aggregated about the ostiole, 35×2.5–3 μ, about a dozen in number, rather coarse, irregular, simple, and brown. Asci rather short and stout, sporiferous to the small, short, rather abruptly narrower base, distally rounded, 28×10 μ; ascospores hyaline, the septum median with a very slight constriction, or the basal segment slightly shorter and narrower, subdistichous, 12–14×4.5–5 μ.

On the elytra, legs, etc., of a cucujid beetle found in decaying vegetable material, Corral, Chile.

A single specimen of the peculiar host which bears this fungus was collected in decaying vegetable material at Corral. It is evidently a beetle of somewhat unclean habits, since it bears numerous stalked mites, and is covered with a thin film of foreign matter such as one often sees on species of Silphidae. The perithecia are numerous, and appear under a hand lens as black points scattered irregularly over the surface (fig. 1), the individual perithecia nestling among the peculiar hooked spines of the host as shown in fig. 3, and associated with a variably developed, brown, thin mycelium of thick-walled branching hyphae (fig. 2), which can hardly be called a byssus. The terminal hairs eventually break off, exposing the evident ostiole in old specimens. The surface is slightly roughened, and occasionally a hair may be seen projecting apart from the group about the ostiole.

I have concluded with reluctance to apply a new generic name to this type, yet its close relationship to other genera does not seem at all clear. It probably is not truly entomogenous, deriving its nutriment directly from the living insect, as in all the other types herewith described; and it is not unlikely that it may obtain its necessary materials from the film of foreign matter which covers the surface of its host.

Termitaria, nov. gen.—General habit disciform, applanate or hysterioid, orbicular or variously elongated according to position of growth, sessile; consisting of a basal pseudocellular layer,

from which firmly coherent, simple, parallel sporogenous elements arise vertically, forming an even hymenial surface, the contents of the upper portion of each element becoming separated to form a single row of endogenous, simple, hyaline spores, which are discharged through a terminal perforation; the peripheral elements sterile, dark, indurated, forming a well defined rim or exciple; the margin in contact with the substratum slightly spreading and lichenoid.

This structure, which characterizes the mature condition of this very remarkable type, two species of which have been examined from living Termites, appears to be a secondary development, which results from the vertical proliferation of a primary stage similar to that represented in figs. 6 and 13. This primary condition may be more or less elongated or orbicular, varying to some extent according to the position of growth; it is formed by a continuous layer of slightly brownish cells, the whole reducible to a copiously branched and septate filament, the branches of which are in lateral contact, the ultimate branchlets forming a radiate lichenoid margin. As the cells mature and enlarge, there may be more or less displacement, as a result of which the fundamental arrangement of the cells in branching filaments may be obscured or obliterated. The general appearance of this stage, as represented in the figures cited, recalls that of some species of *Asterina* or of a young *Aglaozonia* or some species of *Coleochaete*, the resemblance to the latter being rendered more realistic by the presence of the projecting bristles of the host, which are completely surrounded by the advancing margin and are left projecting from the thallus without displacement. Of the cells which form this primary incrusting layer, many usually become characteristically modified (fig. 13), assuming the appearance of chlamydospores, which are clearly differentiated from the unmodified cells about them by their greater size, thicker walls, more rounded outline, and deep brown color. Whether these bodies are ever separated and become functional spores it has not been possible to determine, although various instances have been seen in which they appear to have been dislodged.

The preliminary stage just described has been seen in only a few cases, and a complete series, showing the transition from this to the

mature condition, has not been obtained. From such young specimens as have been examined, however, it is evident that a proliferation takes place over the surface of the primary stage, which results in the development of the structures distinguishing the genus. The primary thallus thus forms a thin substratum, more or less firmly coherent to the surface of the host, on which the secondary stage is seated, and which is clearly distinguishable both in crushed specimens and in sections; the brown chlamydospore-like cells persisting *in situ*, singly or in groups.

A section of the mature fungus, which under a hand lens has the appearance of a black *Hysterium* when growing on the legs (fig. 7), or of a small discomycete with pale hymenium and black margin on other portions of the host (figs. 8, 9), shows a differentiation into several distinct regions. The first is a thin dark layer of cells, in which many or few of the chlamydospore-like bodies may be visible at intervals, and which, in a favorable section, may include the primary attachment of the fungus, an indentation, associated with a group of dark cells (fig. 14) opposite which the hypertrophied cells of the host are usually somewhat brownish. No indication has been seen of any actual penetration of the parasite through the integument of the host; but these primary attachments are readily distinguished, and usually appear as a limited dark area which shows through the sporogenous region when the fungus is viewed vertically, as in fig. 9.

Above this primary layer, and derived from it by vertical proliferation, is a region of irregularly polygonal, hyaline cells, the origin of which, as components of a series of branching hyphae, is obscured or quite obliterated through unequal growth and mutual pressure, and is only indicated by a tendency of the lower cells to retain an arrangement in vertical rows. The thickness of this region is somewhat variable, the cells becoming smaller and numerous above; the uppermost giving rise to the straight, erect, tubular, and apparently always simple filaments which compose the sporogenous layer or region. In this layer, which is four or five times as thick as that from which it is derived, two regions are again recognizable, the limits of which may be very clearly indicated. In the lower of these regions the continuous protoplasmic content

of the individual filaments is more dense, and stains more deeply; although this distinction becomes less marked in older individuals, in which, however, the limits of the zone may be even more clearly marked (fig. 14) through the often deep distal suffusion of the walls. Above this line of demarcation in the upper zone, which simulates an ascigerous hymenium, the walls of the upright tubes become somewhat thicker, gelatinous, and tenaciously coherent; while the protoplasm of each is segmented to form a series of short cylindrical spores, which is constantly renewed and pushed upward by the activities of the denser contents of the lower zone. The spores separate from one another as they pass into a somewhat paler region below the surface (fig. 12), becoming slightly rounded at the extremities, with a few sometimes conspicuous granules. The discharge of these endogenous spores through the terminal perforation of the tube has not actually been observed, but is doubtless effected with some violence, the thickened walls around the opening, and the mutual pressure of the gelatinous hymenial elements, combined with the constant pressure from below, affording an effective mechanism for this purpose. The dimensions of the sporogenous elements are very small, and owing to their gelatinous nature it is usually only with the greatest difficulty that the limits of single tubes can be distinguished with exactness in sections, or in crushed specimens; in fact no outlines are clearly defined in this region, and even after staining, the minute spores are often recognized with difficulty under high magnifications. The spores do not seem to possess a wall, or if they have one it is so thin as to be hardly demonstrable. Isolated spores are seldom recognizable on the hymenial surface of healthy individuals, but when the host is confined for a considerable period under somewhat unfavorable conditions, the normal discharge seems to be interfered with, and it may become whitish with a coating of extruded spores.

At the periphery of the hymenium the sporogenous tubes become sterile, thickened, and blackened, forming the inner portion of the well defined, deep black-brown rim or exciple; while a narrow, radiate, lichenoid margin spreads out externally from the base (figs. 7, 8), in close contact with the surface of the host.

As far as can be determined from the series of specimens examined, there seems to be no continuous increase of the fungus in diameter after the original proliferation of the primary stage, which gives rise to the sporogenous region. This is indicated by the fact that this region, as soon as spore formation has begun, is surrounded completely by sterile indurated structures, incapable of radial extension, and also by the fact that the bristles of the host, which are surrounded by the filaments of the preliminary stage, are not bent down as by an advancing margin, but retain their normal position, and may even be seen projecting beyond the hymenial surface of mature individuals, as in fig. 10.

Although each individual must produce an enormous number of spores, this very curious type does not appear to have been very successful in propagating itself effectively; for although its hosts are densely gregarious and live under conditions which should be very favorable for the communication and development of such parasites, hardly more than 1 per cent of the individuals in an infected nest appear to bear the fungus. SNYDER, who was the first to observe the type species of this parasite and to whom I am greatly indebted for the original material examined, informs me that he has found this ratio of infection more or less constant in material from a number of different sources, and SMULGAN, who has also kindly communicated material from the Boston region, makes a similar estimate. In the case of the second species, described from the Island of Grenada, I have also found almost exactly the same percentage of diseased individuals among the several thousand hosts examined.

It does not appear seriously to inconvenience the insect on which it grows, and the only indication of injury is a slight browning of the tissue immediately opposite the primary attachment, as shown in fig. 14, although all the cells of the tissue lying immediately below the integument are hypertrophied, wherever the fungus is in contact with the host, often assuming a rather regular palisade-like structure, similar to that shown in fig. 10. It is most conspicuous when growing on the abdomen (fig. 9), where it is likely to assume a more regular and rounded form, being suborbicular, or more often transversely elongated, with an even or sometimes

slightly irregular outline (fig. 8); but it may also attack the thorax and head, and very often occurs on the legs, where it assumes a long fusiform outline, like that of a hysterium (fig. 7). Individuals of the latter type which have developed on the tibia, from a point of infection near the terminal claws, are sometimes connected with the original point of infection by a narrow primary thallus which remains unchanged on the intervening joints of the leg, spreading out and producing the secondary stage only on the broader and more nutritious tibia.

The relationships of this fungus are quite obscure. The general characters of its primary stage might suggest a resemblance to some Asterinae, or to a similar incrusting type. Its mature condition, however, evidently a Fungus Imperfectus, seems to give it a formal place among the Leptostromaceae. Its method of sporulation, which in certain respects recalls that of the Chalareae, or of *Sporochisma* or *Endoconidium* among the Hyphomycetes, would seem to make its position in this group an isolated one.

Termitaria Snyderi, nov. sp. (figs. 13–17).—Characters of the genus. Sporogenous filaments with blunt or flat perforate terminations forming an even hymenium. Total thickness of sporodochium 70–80 μ; basal region including primary thallus 18–20 μ; sporogenous region 55–65 μ, the upper zone 25–28 μ; sporogenous hyphae a little over 3 μ in diameter; free spores about $3.5 \times 2\ \mu$. Sporodochium on abdomen 400×400–1000 μ.

On workers and soldiers of *Reticulitermes flavipes* and *R. virginicus*, Washington, D.C., the former also vicinity of Boston. On *Reticulitermes*, nov. sp., California. On *R. lucifugus*, Sardinia. A specimen on *Rhinotermes marginalis* from Turkeit, British Guiana, kindly communicated by NATHAN BANKS, does not appear to differ from the type. The material, however, is too scanty for a satisfactory determination.

This form, which is evidently widely distributed, was first observed by SNYDER, to whom I take pleasure in dedicating it, and who has figured its gross appearance in *fig. 9c*, p. 29, Bull. 94, Part II, Bureau of Entomology. It was first sent me at his request by A. D. HOPKINS with an inquiry as to its possible fungus nature, and has also been brought to my laboratory by both SNYDER and SMULGAN from the Boston region.

Termitaria coronata, nov. sp. (figs. 6–12).—Sporogenous hyphae bearing distally a crown of several, more often four, brown-tipped,

minute, pointed prolongations which form a minutely echinulate hymenial surface. Total thickness 80–100 μ; basal region including primary thallus 16–20 μ; sporogenous region 70–78 μ, its upper zone 45–50 μ; sporogenous hyphae $\times 2.5\,\mu$; spores about $3.5 \times 2\,\mu$.

On *Eutermes morio* var. *St. Luciae*, Grand Etang, Grenada, B.W.I.

The two species described, although hardly distinguishable in general appearance, seem to be clearly separated by the minute, dark, toothlike projections which terminate the sporogenous hyphae in *T. coronata*, and give to the surface of its hymenium a finely punctate appearance which is suggested with sufficient exactness by the stipple in figs. 7, 8, and, under a high power, has the appearance represented in fig. 11. In *T. Snyderi*, on the other hand, the corresponding terminations are unarmed, blunt, and when viewed from above show clearly their rounded ends, slightly polygonal from mutual pressure, and having a readily distinguishable central pore (fig. 15).

The dimensions of the two species, although they are variable in either case, are usually somewhat different; the sporogenous hyphae of *T. coronata* being slightly larger in diameter and length, the relative length of the portion included in the upper zone always being greater. The extremities of these hyphae in this species are quite hyaline and gelatinous, and so tenaciously coherent that I have been unable either to distinguish clearly the terminal pore, or to trace definitely to their bases the characteristic terminal toothlike prolongations shown from above in fig. 11, and laterally in fig. 12. While within the tubes the spores are evidently compressed, and when free increase in diameter, becoming more rounded at the extremities.

Muiogone Medusae, nov. sp. (figs. 18–25).—Sporophores about as long as the spores, rather closely septate, densely crowded so that the whole forms a cushion-like mass on the surface of the host. Spores somewhat irregular, subpyriform, distinctly broader distally, uniform pale dirty brownish, consisting of 10–12, more often 11, more or less regular tiers, the numerous cells of which may be slightly misplaced, those of the basal and distal tiers often slightly larger than the rest, but otherwise indistinguishable from them; a variable number of the distal ones proliferating while still quite young to form a terminal group of tapering, spirally coiled, simple or sometimes once branched appendages which may bear minute secondary spores at their pointed extremities or on short, pointed, subterminal branchlets. Spores $38–45 \times 20–24\,\mu$; terminal appendages $28–30 \times 4\,\mu$ at base; stalks, maximum, $38 \times 6\,\mu$.

On the under surface of the abdomen of *Chromopterus* sp., Kamerun, West Africa.

The fly on which this curious form grows is closely related to, if not identical with, *C. delicatulum*, which bears the type species of *Muiogone*. It is quite unexpected that a genus, which has not been seen on any of the numberless genera and species of flies from the tropics that I have examined, should be represented on the same, or on two at least very closely related hosts, by two such clearly distinguished species, of which but one specimen in each instance is known. The present form, although it has exactly the same gross habit, and occurs in the same position on the underside of the abdomen, is clearly distinguished by its uniform pale brown color, the sometimes total absence of any suggestion of a distinction between basal distal and median regions in the somewhat more irregular cell-tiers, and especially in the terminal, spiral, septate, tapering appendages which replace the short spines of the type species, and the resemblance of which to a Gorgon's head has suggested the specific name. These appendages are not formed after the spore has matured, but begin to appear some time before it has attained its full size (fig. 22), although most of the cell divisions have been completed. There is some variation in the spirals, which may be quite regular, or rather indeterminate; and although they usually end in a pointed apex, they may be somewhat blunt. The minute secondary spores are only recognizable here and there in spores which are still *in situ* (figs. 23, 24). The primary spores become detached, together with an adherent portion of the stalk, and there seems to be no definite mechanism for abjunction. After having been broken thus, the base of the stalk, which remains in position, proliferates as shown in figs. 18 and 19, so that the spore mass is constantly renewed. Owing to the presence of the terminal appendages, as well as the lack of any clear differentiation between the basal, terminal, and middle regions, the original generic diagnosis should be slightly modified.

Muiaria curvata, nov. sp. (figs. 26, 27).—Sporophores and sterile elements springing in small numbers from a compact blackened base. The spores 2 or 3 in a group; the stalks short, of 5 or 6 cells; the termination rather slender, strongly curved, or characteristically recurved distally; the body of the spore rather clearly distinguished, marked by large, very irregular, more or less longitudinal patches, separated by fine light lines and slightly roughened, the 4 tiers of functional cells rather well defined, including the broadest portion and with convex margins, the cells relatively large; the lower of the 3 cells above, and usually the upper of the 2 or sometimes 3 cells below, showing one longitudinal septum; rather pale yellowish olive brown, the concave side of the termina-

tion darker. Body of spore about 52–60×20 μ, the termination 65–70×8, the stalk 50–65 μ.

On the superior tip of abdomen and wing of a small drosophilid fly, Bocas del Toro, Panama (*Rorer*), no. 2525.

This species is perhaps more nearly allied to *M. repens* and the succeeding species. From the former it is distinguished by its 4 clearly defined functional tiers, its much longer, slender, curved termination, and the absence of an appendage from the stalk; while from the latter it differs in its smaller size and quite differently shaped spores. One other American species, also allied to *M. repens*, is known from Trinidad, but more material is desirable before it can be described.

Muiaria fasciculata, nov. sp. (figs. 28, 29).—Tufts compact, the spores and rather numerous sterile elements arising from a usually well defined black base; the stalks relatively long, the termination relatively slender, and usually curved, but somewhat variable, the body of the spore blackish brown, roughened by very irregula intricate darker markings, the 4 functional tiers well defined, relatively short, paler, and rather abruptly narrower than the cells immediately below, of which two are usually flattened, and one or both longitudinally septate; the cells above 3 or 4, the lower usually septate. Body of spore 85–100×24–28 μ, the stalk 100–210 μ, the distal termination 50–64×8 μ.

On a dull brown drosophilid fly, no. 2749, Kamerun, West Africa.

This species occurs on the wings, especially on the veins, of its host, a rather large smoky drosophilid, several specimens of which have been found to bear it. It is clearly separated from the preceding species by its greater size and different shape. From *M. Lonchaeana*, which is the only other form with which it might be confused, it is distinguished by the fact that the stalk and distal portion of the spore are not roughened, as well as by its different form.

Aposporella, nov. gen.—Mucedinaceous, aposporous, entomogenous, a well defined septate axis attached by a blackened foot and bearing short branches at the septa, which separate short undifferentiated segments distally that are constantly renewed.

Aposporella elegans, nov. sp. (figs. 30, 31).—Axis stout, erect, straight, or but slightly curved, tapering, simple, the superposed cells but slightly longer than broad, hyaline, the black foot clearly defined; the branches short, simple, one to several in an irregular whorl from all but the terminal cells; somewhat appressed, or but

slightly divergent, externally edged with blackish brown, except at the tips; the termination of the axis hyaline, slender, projecting, without branches. Total length 200–540 × 8 μ near the base, where the cells are 10–14 μ long. Branches before breaking, longer, 50 × 4.5 μ.

On the wings of a small fly, Kamerun, West Africa, no. 2645.

Sufficient material of this graceful form has been examined to convince me that the individuals figured are fully matured, and that there is no abjunction of definitely differentiated spores, a character in which it agrees with a small assemblage of aposporous Hyphomycetes of which I have half a dozen or more species from Africa and the East and West Indies that are reserved for future consideration, and to which reference was made in my former paper (*loc. cit.*, p. 237).

In this connection it may be mentioned that SPEGAZZINI has recently (*loc. cit.*) described certain Argentine forms which he refers to *Chantransiopsis*, several dubious examples of which, from Africa and the West Indies, I have myself encountered since the genus was established. One of the forms described by SPEGAZZINI under this name, but which seems to me not closely related to it, is a problematical type which I have examined on Forficulae and Staphylinidae from the East and West Indies, and from Argentina. It consists of a deep brown, several-septate body, resembling a spore of *Hendersonia* for example, elliptical in outline, convex above, and flat below, where it is in contact with the substratum. From usually the end cells of this body are developed a group of simple, straight, septate, hyaline hyphae. I have never seen these hyphae producing anything in the nature of a spore, although SPEGAZZINI figures one which appears to be developing as a terminal proliferation. The position and history of this singular form must, I think, remain somewhat doubtful. Although I have examined hosts well covered with the brown, septate, primary structures described, I have never seen any that suggested their origin and development, which has led me to suspect that they might after all prove to be spores of some fungus, not entomogenous, which develop in situations frequented by the hosts, and adhere to them as the spores of agarics and other Basidiomycetes adhere to Endomychidae and Erotylidae. The peculiar form of these bodies, however, and their almost universal germination in the manner described, make such a supposition doubtful.

In the same paper SPEGAZZINI has described a true species of *Chantransiopsis* which he refers to a new subgenus *Asteronycha*, based on a slight difference in the form of its dark attachment. In his comments on these plants he appears to have misunderstood my expressed opinion in regard to their position, or at least overlooked my statement, on page 230 of my former paper, that the genus "comprises species belonging to the Hyphomycetes," and on page 247, where I mention, in connection with the suggestion that they may be related

to the Florideae or the Laboulbeniales, that "there seems not the most remote possibility that such is actually the case."

Coreomycetopsis, nov. gen.—Axis consisting of an indeterminate series of superposed cells, the basal one modified to form a characteristic foot attached to the host; the distal portion transformed into a sporogonium, its successive septa being destroyed, or absorbed, through the upgrowth of sporophores which spring endogenously from numerous divisions of an intercalary cell, and abjoint terminally simple hyaline spores; which, after being set free in the sporogonium, are discharged through a terminal perforation.

Coreomycetopsis oedipus, nov. sp. (figs. 32, 36).—Nearly hyaline or faintly yellowish, the foot large, strongly concave externally, pointed below, its insertion flattened, wholly concolorous with the remaining cells. Axis usually bent strongly outward above the foot, consisting of 10–15 cells, including the latter; the sixth or seventh from the apex becoming proliferous, after dividing to form a central subpyriform cell and numerous small lateral ones, which are obliquely separated, and grow up through the lumina of the 5 or 6 cells above, abjointing terminally long oval spores somewhat pointed at the base; the cells above, and including the proliferous cell, transformed into a straight symmetrical sporogonium, clearly differentiated, and fusiform or obclavate in outline, broader than the 4–6 subequal stalk-cells which connect it with the foot. Total length 100–135 μ. Sporogonium 45–60$\times$12–15 μ; stalk 10 μ; foot 25$\times$12–15 μ; spores 8–9$\times$2–2.5 μ.

On the tips of the legs of *Eutermes morio* var. *St. Luciae*, Grand Etang, Grenada.

This form is usually solitary, attached to the terminal joints of the legs, and from its pale color is not readily seen, although it is larger than many Laboulbeniales. Its remarkable analogy to *Coreomyces* is suggested by the generic name selected, and if the spores were formed in asci, instead of being abjointed, it would be placed near that genus, since the history of development of its sporogonium, and that of the perithecium in *Coreomyces*, is remarkably similar. The destruction of the upper cells to form the common cavity of the sporogonium does not appear to be due wholly, at least, to the upward pressure of the traversing sporogenous elements, since these cells evidently begin to disorganize as soon as the first intercalary divisions appear

(figs. 33, 35), and the uppermost septa are not reached by the sporiferous filaments themselves.

In general appearance this plant is so like some of the Laboulbeniales that at first I was inclined to believe that it might prove to be the male individual of some ascigerous form characterized by an entirely new type of antheridial structure. Its development, however, is so widely different from anything hitherto known among the Laboulbeniales that there seems to be no good reason to suppose, in the present condition of our knowledge of such parasites, that it is even remotely related to them, an opinion which is supported by the fact that a careful search has failed to bring to light individuals of a different nature. Since, however, its relation to other types of fungi is equally problematical, it will have to await further developments in the limbo "genera incertae sedis," in company with its companion *Laboulbeniopsis* on the same host described below, to which, despite a superficial similarity, it seems also quite unrelated.

THAXTERIOLA Spegazzini.—This name has been used by SPEGAZZINI (Ann. Soc. Nat. Arg. **85**:314) in a paper entitled "Observaciones Microbiôlógicas," under the caption "Anforomorfideas Argentinas," to designate a series of very minute and simple forms common on various insects, especially Staphylinidae, two species of which were figured in my former paper (*loc. cit.*, figs. 30–31), and referred to in the text (p. 250), no name being used to designate them, owing to a lack of any complete knowledge of their history and to their general insignificance. These organisms consist primarily of two cells, the lower attached by a well defined black foot, corresponding entirely with that of most Laboulbeniales; while the upper, having become prolonged to form a necklike termination, and having previously separated, at its base, a smaller cell from which it is more often obliquely distinguished, produces minute, naked, sporelike bodies formed in a single series and discharged through the perforate extremity. These plants closely resemble male individuals of *Amorphomyces*, among the Laboulbeniales; but their occurrence in large numbers, and under no other form, precludes the possibility that they may be conditions, or stages, of any member of this family. Whether, as in the sperm cells of *Amorphomyces*, the spores produced by *Thaxteriola* are formed continuously, as seems most probable, or cease to be produced after the protoplasm of the sporogenic cell has been exhausted, I have not been able to determine satisfactorily. SPEGAZZINI, how-

ever, since in his generic diagnosis he says that "articulum supremum sporis amoeboidëis repletum," appears to assume that the latter supposition is correct. I have not seen the sporogonium "sporis repletum," and the usual appearance of the individuals examined has been that shown in figs. 37, 38, the spores occupying the upper portion of the cell and being arranged in a single series, not irregularly disposed as in SPEGAZZINI'S fig. 5, and similar to that which occurs in the closely related *Endosporella* described later. It should be pointed out, however, that in the genus *Laboulbeniopsis*, a description of which follows, and which appears to be otherwise similar, a simultaneous formation of irregularly distributed spores appears to take place.

In order to facilitate a direct comparison between this type and the others here considered, I append a description of a Javan form that seems sufficiently distinct for ready recognition. Since they are now known to occur on such diverse hosts as gamasid mites, Forficulae, Hemiptera, and Coleoptera, it may be assumed that numerous species of this group exist, none of them too well defined; and it is probable that by the time systematists have finished with them, posterity will have become burdened with a horde of these uninteresting little plants.

Thaxteriola nigromarginata, nov. sp. (figs. 37, 38).—Subsigmoid, pale brownish, except the clear hyaline base and apex; the distal half edged with deep blackish brown, the suffusion broader toward the middle. The basal cell including half the total length; its extremity slightly broader than the distal half, the lower cell of which is very obliquely distinguished from the upper, and is distinctly concave on its longer side, being also free from any blackish suffusion. Total length 62–68 μ; greatest width (distal portion of basal cell) 8–8.5 μ.

On the hairs of a minute staphylinid, no. 2082, Samarang, Java.

I am indebted to JACOBSON for the host bearing this species, which was found among a few beetles collected at Samarang. It seems sufficiently well distinguished from the types usually common on Staphylinidae by its slightly sigmoid outline, more slender distal half, the lower cell of which is distinctly concave on one side when viewed laterally, by the very oblique separation between this and the sporogenous cell, and by the well defined and rather clearly circumscribed black marginal suffusion of the latter, which contrasts strongly with the adjacent hyaline areas.

Two species of this genus have been described by SPEGAZZINI, to one of which, *T. infuscata*, he refers the form represented in fig. 31 of my former paper, which represents an individual found on *Labia minor* in Cambridge, and is distinguished by the fact that the two upper cells are not separated by an oblique septum. His second species, *T. subhyalina*, which occurs on *Aphodius*, is said to be distinguished by the fact that it is always hyaline, the neck more strongly curved, and the basal cell relatively shorter.

A second genus of a similarly nondescript type has been named *Entomocosma* by the same author (*loc. cit.*, fig. 7, pp. 312–315). Although possibly related to the present genus, its essential characters are not at all clear. It seems in some respects similar to a problematical type, of which I have material collected at Waverly, Massachusetts, in 1893, on *Tachinus pallipes*, and which I have not subsequently observed.

It is to my mind very doubtful whether any close relationship exists between these genera of "Thaxteriolae," to which two others are added below, and the "Anforomorfas" with which SPEGAZZINI associates them, and of which the *Amphoromorpha entomophila* of my former paper may be taken as the type. As in the case of *Coreomycetopsis*, however, their relationships to other groups are equally obscure, and they must remain among the "genera incertae sedis" until the discovery of further types which may possibly throw some light on their affinities.

Endosporella, nov. gen.—Axis consisting of 4 superposed cells, the basal attached by a well differentiated foot; the terminal one spinose, separating uniseriate endospores distally, which escape through a terminal pore.

Endosporella Diopsidis, nov. sp. (figs. 39–41).—Foot small, black, and pointed; basal cell abruptly narrower and hyaline below, the upper half becoming much broader and somewhat inflated distally, obliquely suffused with blackish brown. Second and third cells much shorter, subequal, or the upper usually slightly longer and broader; terminal cell a sporogonium, sometimes as long as the rest of the individual, deeply tinged with blackish brown, except the hyaline tip, which is primarily spinose and becomes perforate, the upper half or more becoming filled with a simple

series of flattened superposed naked spores, which are successively separated from the protoplasmic mass below. Apex opening irregularly beside the large terminal spine, which seldom persists. Total length 100–150×10–13 μ. Sporogonium 50–60×10–12 μ.

On the terminal claws of the legs of *Diopsis* sp., nos. 2716, 2717, Kamerun, West Africa.

This type is most nearly allied to *Thaxteriola*, from which it differs in being 4-celled, the sporogonium having no differentiated efferent neck, and discharging broad flat spores. A majority of the individuals examined are comparatively young, and only a few are beginning to form spores, so that in this instance it is also impossible to say whether sporulation is a continuous process or ceases after all the primary contents has been used.

Laboulbeniopsis, nov. gen.—Axis simple, consisting of a differentiated foot, a 2-celled stalk, and a well defined terminal sporogonium, at the base of which two cells are distinguished, the rest of the cavity being filled with numerous minute hyaline spores, which escape through a terminal perforation.

Laboulbeniopsis Termitarius, nov. sp. (figs. 42, 43).—Foot and sporogonium pale brownish, the stalk nearly hyaline. Foot large, externally strongly convex, a portion of its flat insertion deeply blackened, more or less pointed below; the stalk much narrower, its upper cell shorter and broader than the lower. Sporogonium as long as or longer than the stalk, straight, subsymmetrical, slightly inflated below, tapering distally to the rather broad, slightly flaring terminal pore, which is subtended by a scarcely distinguishable constriction; the basal cells occupying the lower fourth or less of the cavity, lying side by side, one slightly larger than the other. Total length 100–130 μ; sporogonium 45–50×12 μ; stalk ×8–10 μ; foot 25×12 μ; spores 3.5–4×2.5 μ.

On tips of legs of *Eutermes morio* var. *St. Luciae*, Grand Etang, Grenada, B.W.I.

This form occurs very rarely, associated with *Coreomycetopsis*, of which it was at first believed to be a stage or condition. The two, however, do not seem to be related, although their general appearance is so similar. There is not sufficient material available to determine the complete history of its sporulation. As far as can be determined from the material available, the spores develop simultaneously, filling the whole cavity of the sporogonium above its two basal cells, and there is no evidence in the specimens examined that successive periods of spore-formation occur, after the first are discharged.

In several cases the sporogonium has emptied itself, leaving a few residual spores, and in such individuals the basal cells, as shown in fig. 42, are already more or less disorganized, while the spores may be considerably swollen and rounded, measuring even as much as $6\times3.5\ \mu$, having surrounded themselves with more or less evident walls.

Despite the apparently simultaneous formation of the spores, however, and their irregular distribution throughout the cavity of the sporogonium, it seems best, at least provisionally, to associate this type with *Thaxteriola* and *Endosporella* in a group of "Thaxteriolae," to which the genus *Entomocosma* Speg. may possibly be added.

AMPHOROMORPHA Thaxter.—The type of this genus, *A. entomophila*, was described and figured in my previous paper (*loc. cit.*, p. 251, figs. 26–28), having been observed on species of *Labia* and *Diochus* from the Philippines. It has since been noticed on a carabid allied to *Platynus* from Jamaica, on a species of *Pachyteles* from Verdant Vale, Arima, Trinidad, and on a host allied to *Ardistomis* from Hayti. Although the specimens obtained from these sources correspond in all respects with the original types, the more abundant material thus made available furnishes certain additional information which is of interest and tends to harmonize the characters of this species with those of other related forms which are not distinguished by the same striking specific peculiarities.

An examination of specimens removed *in toto*, so as to include the whole individual, including its attachment, and viewed anteriorly or posteriorly, shows that the foot, which, when viewed sidewise, usually appears to be black and quite opaque and would naturally be assumed to correspond to that of most Laboulbeniales, or of the Thaxteriolae, is of quite a different nature. This is due to the fact that its main mass consists of a secretion which spreads over the surface of the host, and, when viewed in the position indicated, is translucent, and may be transparent enough to show the actual termination of the organism. This termination is very clearly a short, abruptly distinguished rhizoid (fig. 45), which is held firmly against the host by the indurated secretion just mentioned, and suggests the somewhat analogous rhizoidal attachments of some of the Rhizideae among the Chytridiales.

An identical condition is seen in the other species of this type, two of which are illustrated in figs. 44 and 46. The character of

the wall, its general appearance and texture, are also very like some of the Chytridiales, and unlike that of the Thaxteriolae, with which I was at first inclined to associate them. My present impression, however, is that they have little if any relationship to one another. There seems no reason to believe that they are not, like the Chytridiales, strictly unicellular. Although their developmental history is not, as yet, exactly known, it seems probable, from an examination of the stages available, that the sequence of events may be very similar to that seen in the temporary sporangia of *Cladochytrium Alismatis*, for example. On the basis of this supposition the original cell may be assumed to divide completely into spores, as has been the case in the individual of *A. entomophila* (fig. 45). Figs. 26, 27, and 29 of my former paper, on the other hand, may well be interpreted as illustrating different periods in the spore discharge, which may be, in part at least, effected by pressure exerted as the result of an intrusion into the sporogonium of a new sporogenous cell, which may be assumed to fill the cavity after the spores have effected their exit, and to become transformed into another spore mass to be discharged in a similar fashion. As there is no indication that cilia are present on the spores, it is not easy to see how otherwise the sporangium could be completely emptied through so narrow an orifice. However this may be, it is evident from the condition shown in fig. 45 that the generic diagnosis must be modified, no sterile basal cell being clearly distinguished.

It is also evident, however, that the true position of this type, as well as the exact sequence of events in its development, have yet to be accurately ascertained. I should not be reluctant even to turn it over to the zoologists, although E. G. RACOVITRA, who has figured a more simple type observed on crustaceans (Arch. Zool. Exp. 1907–1908. pl. 10. fig. 26; 1908–1909. p. 272. fig. 2), speaks of it as "une Laboulbeniacea parasite." Further references of this nature, if they have occurred within the past few years, have escaped my notice, with the exception of the account given by SPEGAZZINI in the paper already cited, in which he described under the name *Amphoropsis* three species: *A. minuta* on *Hister*, said to be the same as that represented in fig. 29 of my previous paper; *A. subminuta* on *Echiaster*, represented as somewhat more pointed

and sessile; and *A. media*, which is somewhat larger and more distinctly stalked. A second genus, *Myriopodophila*, is also created, with a single species, *M. argentina*, the only basis for which appears to be a slender habit. All of these 4 species are represented in the figures as octosporic, although this character is not mentioned in the text. I should personally be reluctant to separate either of these forms from *Amphoromorpha*, and the species of *Amphoropsis* are certainly congeneric with the types illustrated in figs. 44 and 46. Since the material of the species represented in fig. 44 is sufficiently abundant and has been observed on two different genera of roaches, it seems worth while to append a description, although all the individuals examined are at the same point of development, the sporogonia being completely filled with spores.

Amphoromorpha Blattina, nov. sp. (fig. 44).—Yellowish, sessile, with a large dark foot. Form elongate oval, somewhat broader distally, the apex rounded. Spores between 50 and 100, about 5 μ in diameter. Total length of sporogonium 55–70 × 18–20 μ, exclusive of the foot, which is 18–22 × 18 μ, seen in front view.

On the axis of the antennae of a dark wingless and a pale winged blattid, nos. 2938 and 2939, Grand Etang, Grenada, B.W.I.

This species is similar to *A. media* in size, but differs in its form, its sessile habit, and its much more numerous, smaller spores. It is apparently confined to the axis of the antennae, where it grows among, but not on, the hairs. A second species inhabiting the hairs, and not the axis, was found in the same locality on a different host, and is represented in fig. 46. This form is characterized by a somewhat different shape, its smaller size, and transparent, hardly suffused, foot.

ASTREPTONEMA Hauptfleisch, Ber. Bot. Gesells. **13**:83. *pl. 8.* 1895.—In a paper entitled "*Astreptonema longispora*, n.g., n. sp., eine neue Saprolegniaceae," HAUPTFLEISCH has described a peculiar organism which grows attached to the chitinized end of the rectum of *Gammarus locusta*, consisting of a simple, unicellular, multinucleate filament, attached at its base, and distally producing a series of successively formed spores, or rather of spore mother cells, within which single definitely walled spores are formed, at first uninucleate, and later containing as many as 8 nuclei. These spores are formed in large numbers and are eventually freed by

the disorganization of the mother cell walls. The filament is attached at the lower end, the wall of which is at first thickened, the thickening organizing a well developed and peculiar sucker-like structure, which forms a definite organ of attachment. The walls of the filament mother cells and spores are comparatively thin, although well defined. As the title indicates, this type was regarded by HAUPTFLEISCH as unquestionably belonging to the Saprolegniaceae, with a possible relationship to *Aphanomyces;* the mother cells, despite the absence of any signs of antheridia or of zoosporangia, being regarded as oogonia, and the contained spores as oospores, a comparison being drawn between them and the seriate oogonia, of *Saprolegnia monilifera* DeBary. The author's conception of the type is summed up in his "kurze lateinische Diagnose für diese neue Saprolegniaceae," which reads as follows: "Thallus non racemosus. Una tantummodo ovospora in ovogonio nata, quasi explens ovogonium. Ovosporae plurium nuclearium oblongae, 2–2.6×7–10 μ. Ovogonia terminalia semper simplici serie adnexa, aliud alii, non transfusa. Sporangia incognita. Antheridia desunt." SACCARDO in the *Sylloge* (**14**:446) places this type among the Chytridiales, but neither author appears to recognize the fact that it has any relationship to the Enterobryae, to which it undoubtedly belongs. The only character which might separate it from the type genus *Enterobryus* is found in the presence of definitely differentiated spores, which replace, or succeed, the terminally abjointed segments which are characteristic of all the species of this genus; but whether this character should be regarded as separating the two types generically, or as extending our knowledge of the little known life cycle of the last mentioned genus, it is not at present possible to decide. In the numerous forms of *Enterobryus* which I have examined, none that have been observed growing within the intestine of the host have shown a development of well differentiated spores; although the terminally abjointed segments may be more or less sporelike, according as they are longer or shorter. It does not seem possible, however, to homologize them closely with the spores of the form described by HAUPTFLEISCH, or with those of the new form described later. It is nevertheless quite possible that, as in many cases among the

higher fungi, certain species of the same genus may be sporiferous in a special way, while others are not; or that differences in environment may bring about the sporulation of species which normally reproduce by separated segments only. In the two instances under consideration, for example, the individuals do not, like most species of *Enterobryus*, grow submerged in the more or less fluid contents of the ventriculus, or smaller intestine, in which the food ingested by the host has only partially been digested; and while the species of HAUPTFLEISCH is attached just within the anus, the new form is found growing on the hard external chitinous plates about the opening. As far as the possible food relations of these two forms is concerned, the situation seems to be quite different, since they come in contact with fecal matters only, which might be supposed to exercise a definite influence on their course of development. It should be mentioned, however, that although I have, in one instance at least, obtained abundant material of what appear to be several species of *Enterobryus* growing outside the anus of a *Passalus* from Grenada, B.W.I., none of the individuals, although they are otherwise very similar, show the sporulation which is so conspicuous a feature in the new form to be described.

This form is characterized by the possession of a huge basal cell; its very thick wall often laminate above, filled with a coarsely granular protoplasm, and attached at its base by a well developed sucker-like attachment entirely similar to that of other species of *Enterobryus*. The primary axis is at first continuous (fig. 47*a*), but later a terminal segment of considerable length is separated, and at least one more may be similarly formed, as in fig. 47*b*, in which a terminal scar shows very clearly that a segment of this sort has previously been abjointed. Such a condition, were it found within the intestine, would inevitably be regarded as belonging to some species of the genus *Enterobryus*. After one or more of these segments has been abjointed, and as a result of the activity of the denser multinucleate protoplasm at the end of the cell below the scar (fig. 51), a series of flattened cells begins to be cut off, each of which is supplied with a single large nucleus. Soon after these cells, or spore-segments, have been separated, they become ab-

ruptly compressed, so that above the fourth or fifth cell, as a rule, the series, when viewed edgewise, is thin and flattened, as is shown in fig. 47. The cells appear to be spore mother cells, within which thin-walled, sausage-shaped spores are firmly held by the thickened sheath which surrounds them and is continuous with the wall of the basal cell from which they were originally separated. As far as can be determined from the material available, these spores, which become eventually multinucleate, are separated by the breaking off of the whole or a portion of the series, and are not set free individually, as seems to be the case in the thin-walled species described by HAUPTFLEISCH. What the further history of their development is cannot definitely be stated. It seems probable that the spore groups are ingested by the xylophagous host, together with other spores of fungi which are present on their natural food, and that, separating as a result of the action of digestive fluids, they either pass through a preliminary period of growth attached to the wall of the digestive tract, or, in being voided with the excrement, become attached and develop at the mouth of the anus.

Although this species differs in its very thick walls, and in the form and more or less permanent association of its spores in series, its characters seem to correspond in all essential respects with those which are said to distinguish *Astreptonema*. Fig. 10 of HAUPTFLEISCH'S plate would indicate that his species is characterized by the separation of one or more terminal segments, which precedes the formation of spores. That these may be antheridia, as he suggests, seems quite improbable, and since, as he states, his material was somewhat scanty, it may prove that in this respect as well as in others the two show a very close correspondence. The cytological characteristics seem to be identical. The nuclei in both are large and rather numerous in the primary cell, more so in the denser contents of the distal region, where the spore segments are cut off (fig. 51), there being fewer toward the base, although one seems to be almost always present just above the foot (fig. 49). This foot is entirely similar in both and identical with the corresponding organ of *Enterobryus;* and the spores, although differing in shape and method of association when mature, are produced in a

similar way within mother cells. There can be little doubt as to the generic identity of the two forms, yet their characters are so similar to those of *Enterobryus* that I have preferred to use this generic name, in view of the fact that in no instance has the complete history of a species of this genus been satisfactorily observed.

Enterobryus compressus, nov. sp.—Hyaline to pale dirty yellowish. Basal cell very large and thick-walled, somewhat broader distally, 500–850×28–35 μ, straight or usually slightly curved at the base, attached by a well defined, slightly brownish yellow foot, shaped like an inverted cup, and distinguished by a slight constriction from the basal cell, which bulges more strongly on one side above it. Segments separated from younger specimens about 200×18 μ, their formation followed by the production of spore mother cells which are formed at the distal end of the basal cell, the series above the fourth or fifth cell becoming broad and flat through compression; the cells about 8 μ long by 35 μ broad by 18 μ thick, each containing a single spore which nearly fills the cavity, surrounded by a thick sheath continuous with the wall of the basal cell.

Growing wholly exposed on the anal plates of a large species of *Passalus*, Dominica, B.W.I., no. 2170, M.C.Z.

The unusually thick walls of this species and the coherence of its spore mother cells no doubt are influenced by its aerial habit, as a result of which it may be exposed to very dry conditions. The individuals represented in fig. 47, with two exceptions, are very old, and seem from the broken outline of their distal ends to have already shed a portion of the spore mother cells. In a majority of the sporiferous individuals, however, it is possible to distinguish the scar clearly shown in figs. 50, 51, from which it may be assumed that a segment has been separated, such as is shown in fig. 47*b*. A large number of Enterobryae have also been obtained growing in the same position on a species of *Passalus* from Grenada, which seems to include more than one species, the larger of which resembles the present form in all respects, except for the absence of any sporulating individuals. All of these, although their walls are somewhat thicker than is normally the case, would be referred without hesitation to *Enterobryus*.

The nuclei shown in figs. 48 and 51 are readily observed in the alcoholic material by decolorizing, after staining with Haidenhain's iron alum haematoxylin. The conditions shown are entirely similar to those described by HAUPTFLEISCH, and serve to show that these plants cannot under any circumstances be related to the higher bacteria, as has been suggested. One may admit that

they must be placed among the Phycomycetes; but they appear to occupy a very isolated position, and it is difficult to agree with this author that they have any close relation to the Saprolegniaceae.

In regard to their relation to the host, it may be said that the aerial habit of the present form seems to exclude the theory that these plants are purely commensalists, since they can only come in contact with the voided feces; and this fact, taken in connection with their highly specialized sucker-like attachment, suggests that they may be, to some extent at least, truly parasitic.

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EXPLANATION OF PLATES I–V

The figures are reduced from camera drawings made with Zeiss dry objectives and eyepieces and Leitz water (no. 10) and oil (1/16) immersion as indicated:

Cantharosphaeria chilensis Thaxter

FIG. 1.—Portion of host greatly magnified, showing distribution of perithecia.

FIG. 2.—Mycelium associated with perithecia; 10, 4.

FIG. 3.—Three perithecia among spines on host; D, 2.

FIGS. 4–5.—Ascus and ascospores; 1/16, 18.

Termitaria coronata Thaxter

FIG. 6.—Young individual showing preliminary stage; subdiagrammatic; D, 4.

FIGS. 7–8.—General appearance of mature fungus growing on leg and thorax respectively; former showing blackened primary attachment which shows through hymenium in center; D, 4.

FIG. 9.—Habit of growth on host; ×25.

FIG. 10.—Section of mature sporodochium, showing hypertrophied cells of host below chitinous integument; dark line of primary thallus shown, succeeded by fundamental layer, and sporogenous region, showing two primary zones, in upper of which a further differentiation into two zones is indicated; sporodochium traversed by two hairs arising from integument of host; semidiagrammatic; D, 1.

FIG. 11.—Hymenium seen from above, showing distribution of toothlike projections from sporophores; 1/16, 12.

FIG. 12.—Sporophores with included spores; semidiagrammatic; 1/16, 12.

Termitaria Snyderi Thaxter

FIG. 13.—Portion of preliminary stage, showing margin and chlamydospores; 10, 4.

FIG. 14.—Portion of section of old individual, showing hypertrophied cells of host below slightly intruded primary attachment, blackened primary

layer, with a few chlamydospores *in situ*, fundamental layer, and above it sporogenous region, comprising two zones, lower distally blackened; 10, 4.

FIG. 15.—Sporophores seen end on, showing terminal perforation; 1/16, 12.

FIG. 16.—Sporophores seen in section, showing origin from cells of fundamental layer, spore, and terminal perforation; 1/16, 12.

FIG. 17.—Free spores; 1/16, 12.

Muiogone Medusae Thaxter

FIG. 18.—Young spore developing from proliferous end of old sporophore; 10, 4.

FIG. 19.—Later stage of young spore of third order resulting from proliferation of second order; 10, 4.

FIGS. 20–21.—Older primary spores; 10, 4.

FIG. 22.—Spore showing origin of terminal appendages; 10, 4.

FIG. 23.—Mature spores, appendages bearing a few secondary spores; 10, 4.

FIG. 24.—Portion of appendage with secondary spores; 10, 18.

FIG. 25.—Group of spores in different stages of development.

Muiaria curvata Thaxter

FIG. 26.—Single plant bearing two mature spores drawn in outline; D, 4.

FIG. 27.—Single spore seen in surface view; D, 4.

Muiaria fasciculata Thaxter

FIG. 28.—Single spore seen in surface view; D, 4.

FIG. 29.—Single plant with several spores in different stages of development and numerous sterile filaments; D, 4.

Aposporella gracilis Thaxter

FIG. 30.—Two plants on wing of fly; D, 4.

FIG. 31.—Two branches, one unbroken, other proliferous.

Coreomycetopsis oedipus Thaxter

FIG. 32.—Young individual of unmodified superposed cells; 10, 4.

FIG. 33.—Young individual, division beginning in an intercalary cell; 10, 4.

FIG. 34.—Mature individual in which septa above intercalary cell have disappeared, forming continuous cavity within which spores are being abjointed; 10, 4.

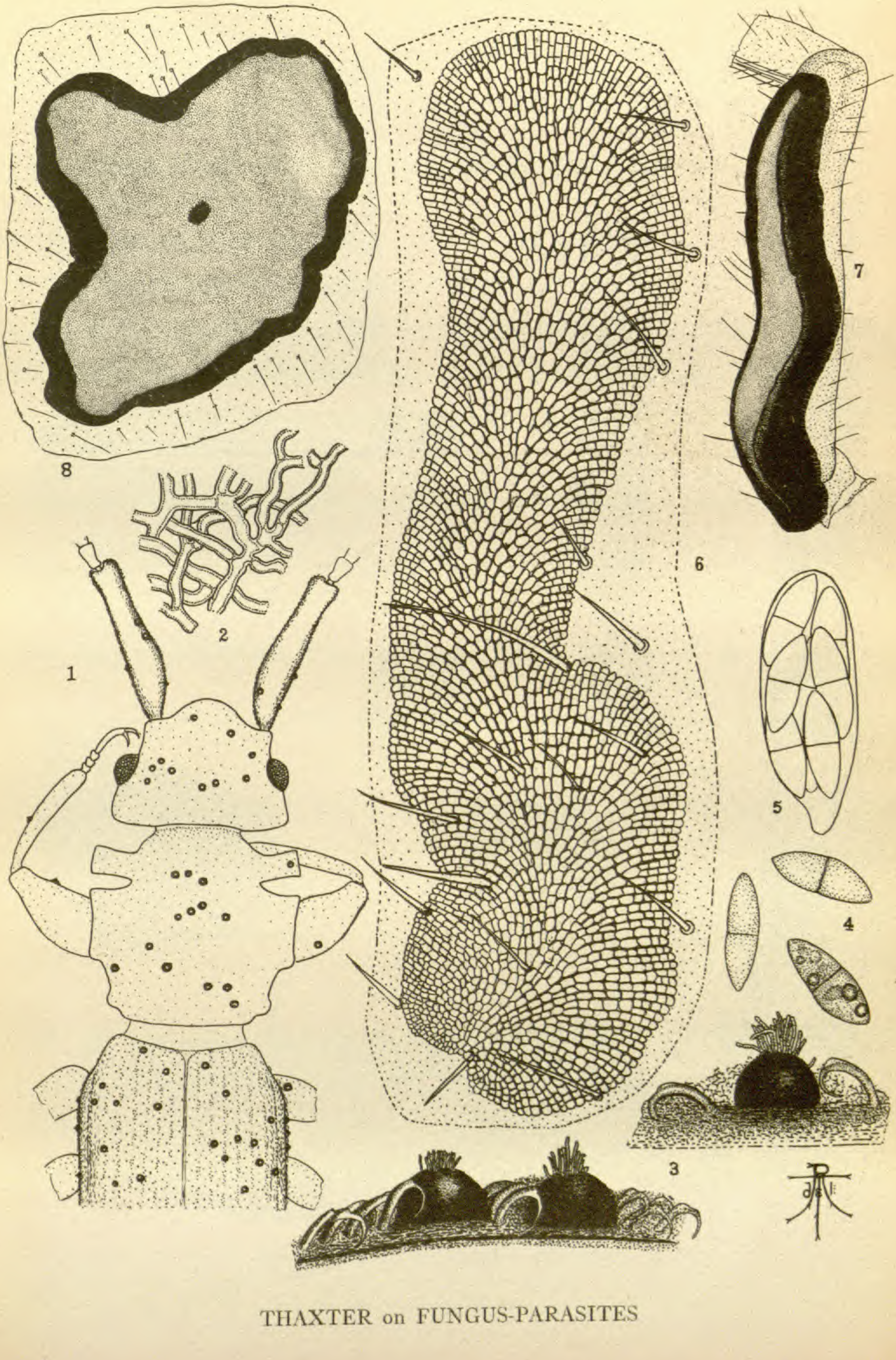
FIG. 35.—Younger individual in which terminal cells are beginning to disorganize, 4 septa still remaining above sporogenous hyphae; 10, 4.

FIG. 36.—Spores separated by crushing; 10, 12.

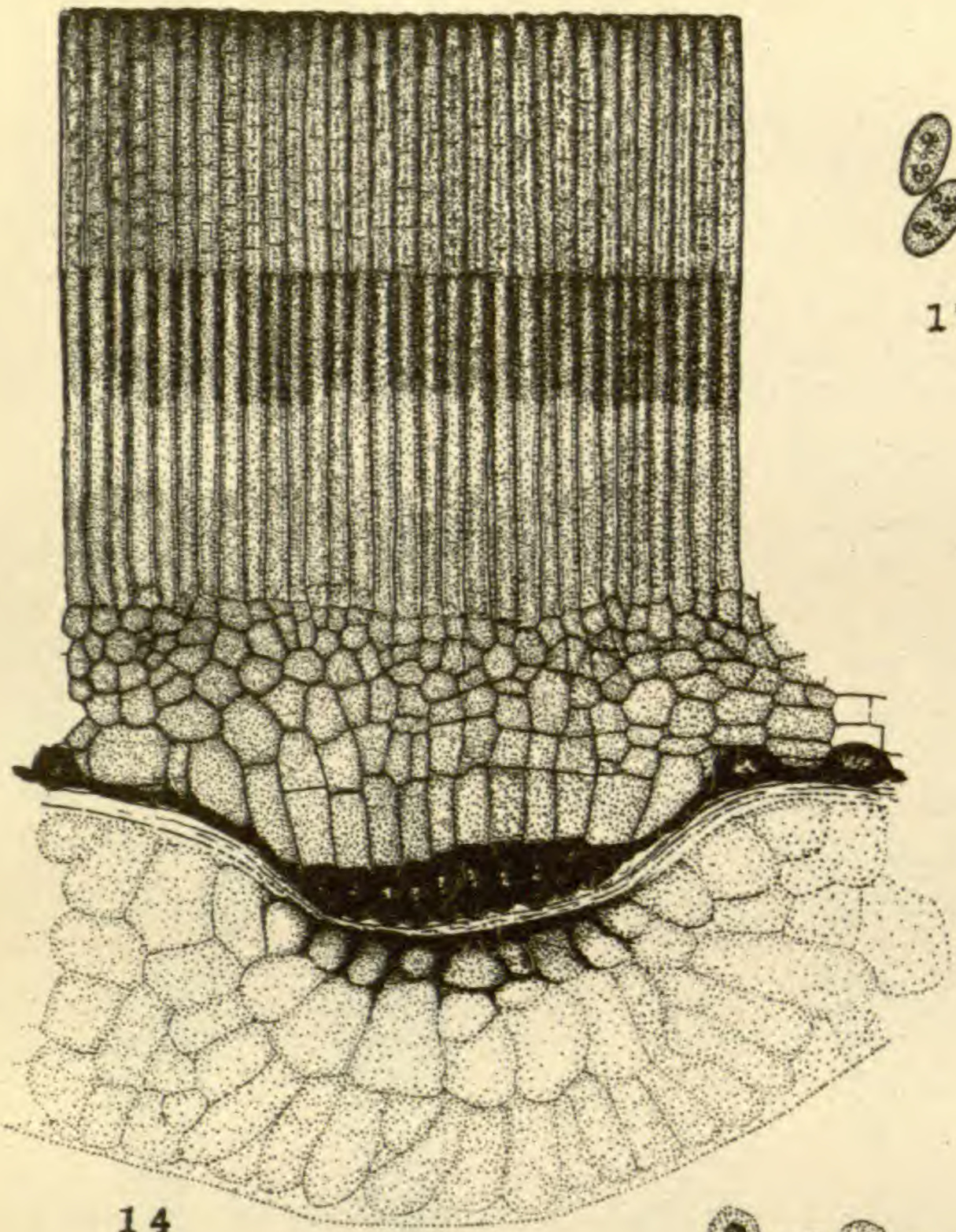
Thaxteriola nigromarginata Thaxter

FIG. 37.—Mature individual; 10, 4.

FIG. 38.—Two individuals *in situ* on spine of host, left one turned to show partly posterior view; 10, 4.



THAXTER on FUNGUS-PARASITES



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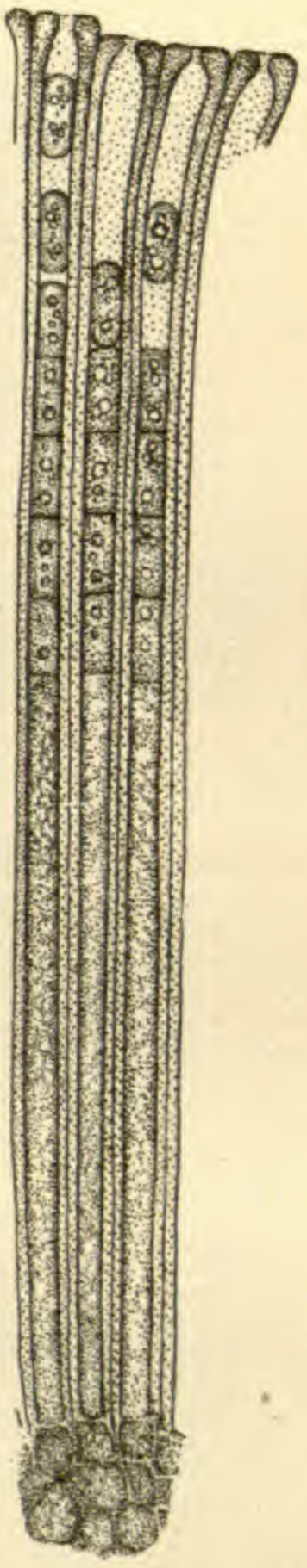

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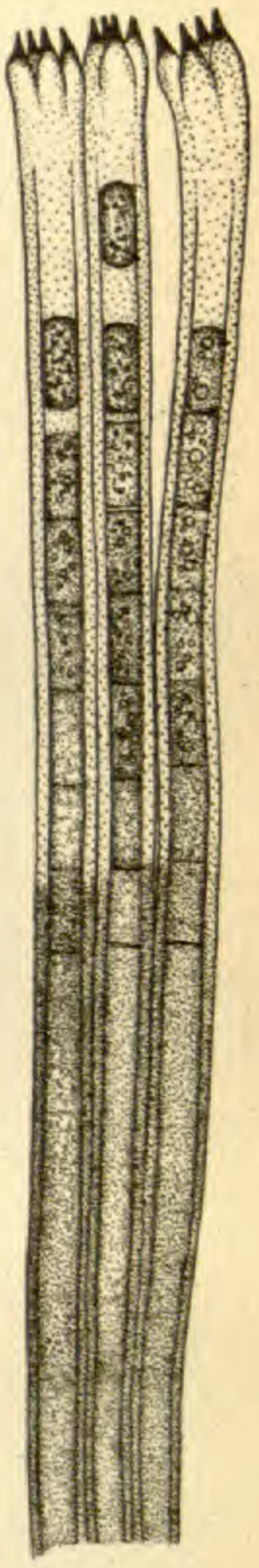

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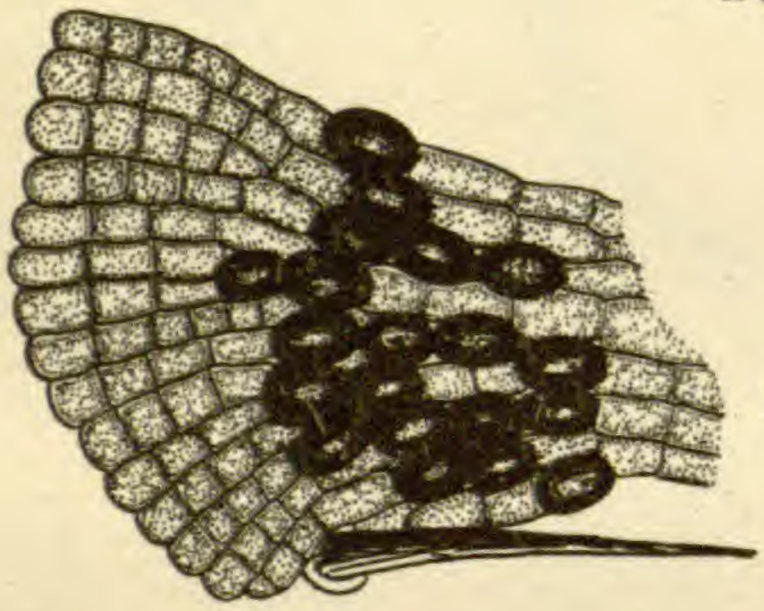

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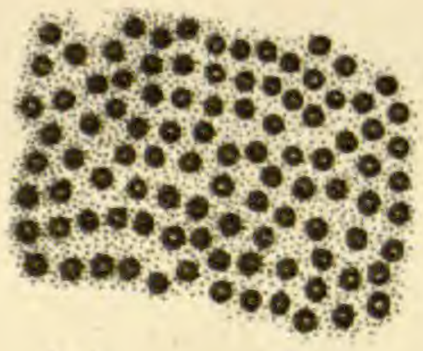

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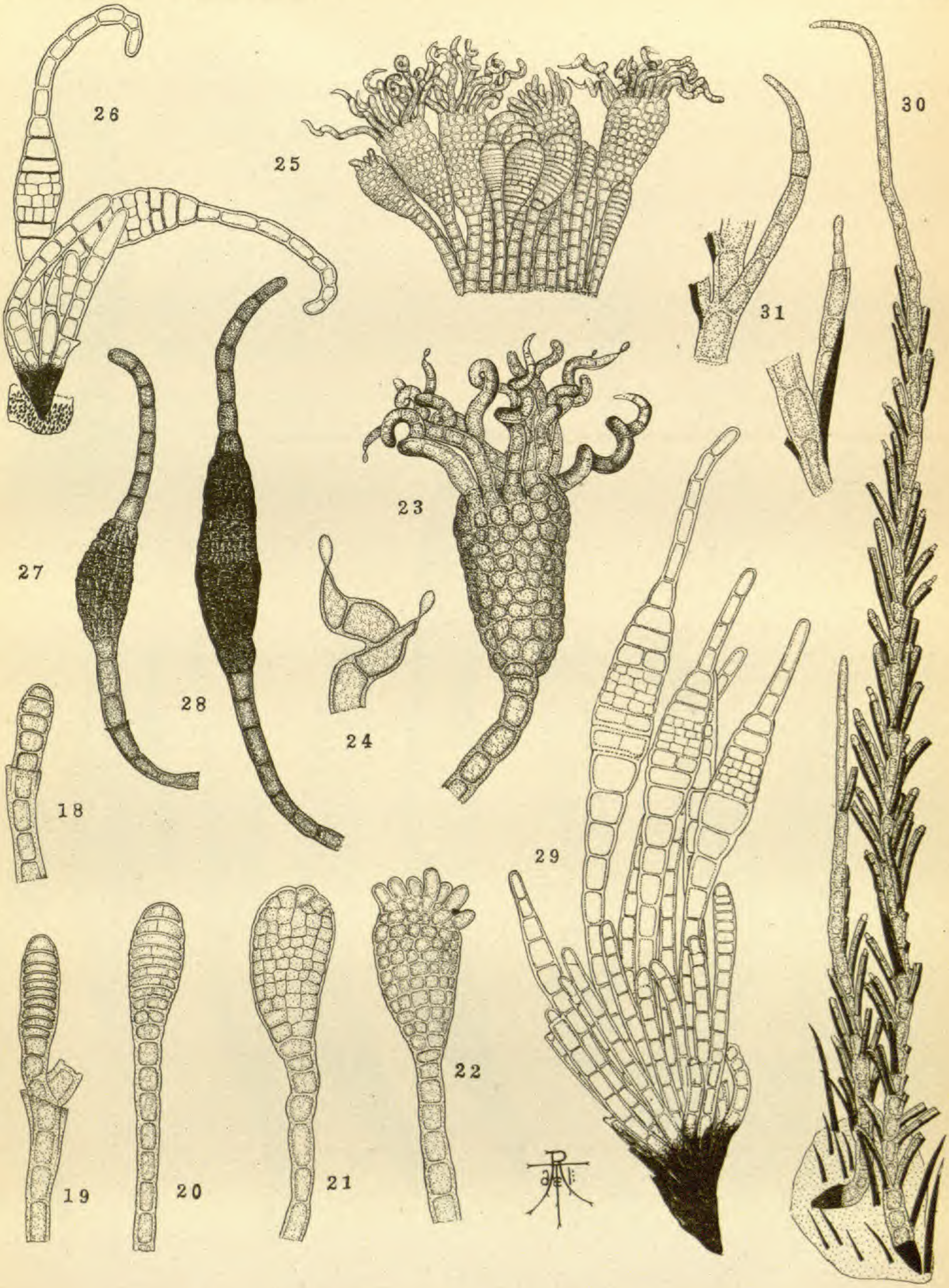
THAXTER on FUNGUS-PARASITES



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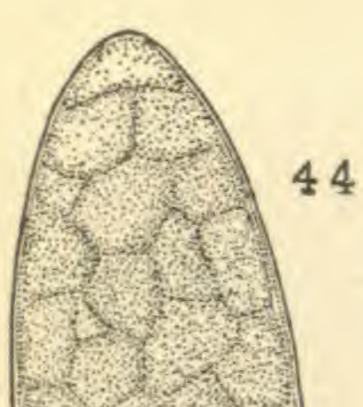

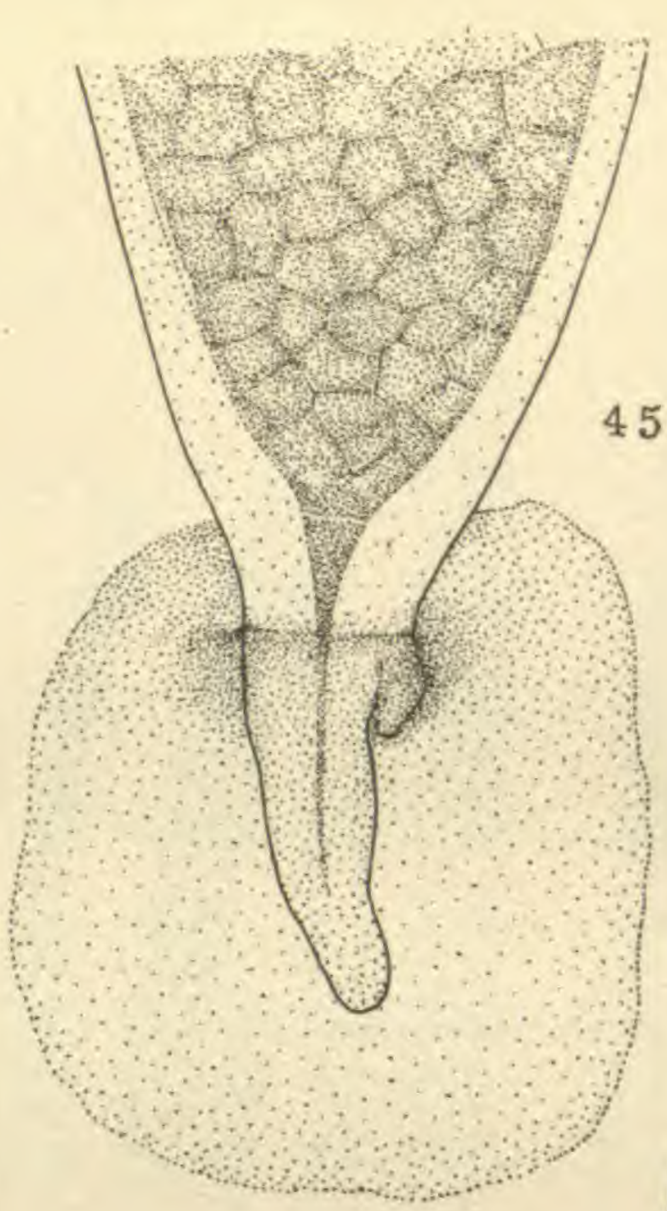

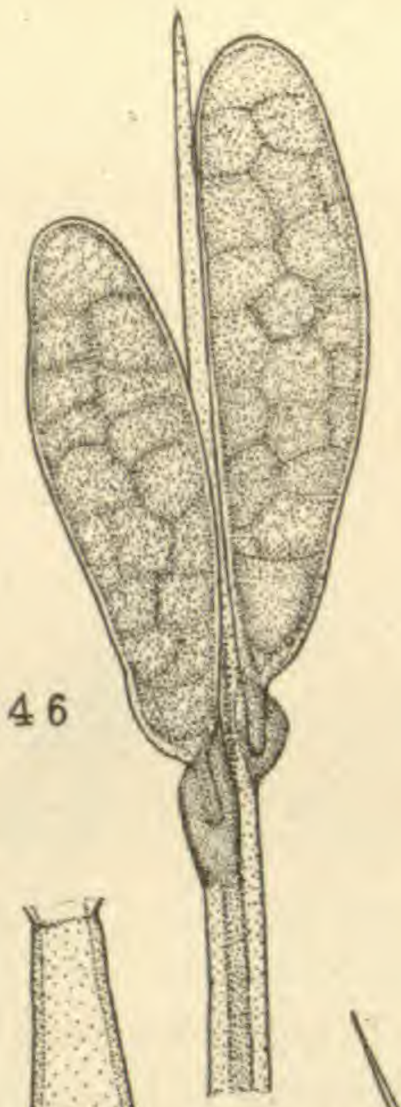

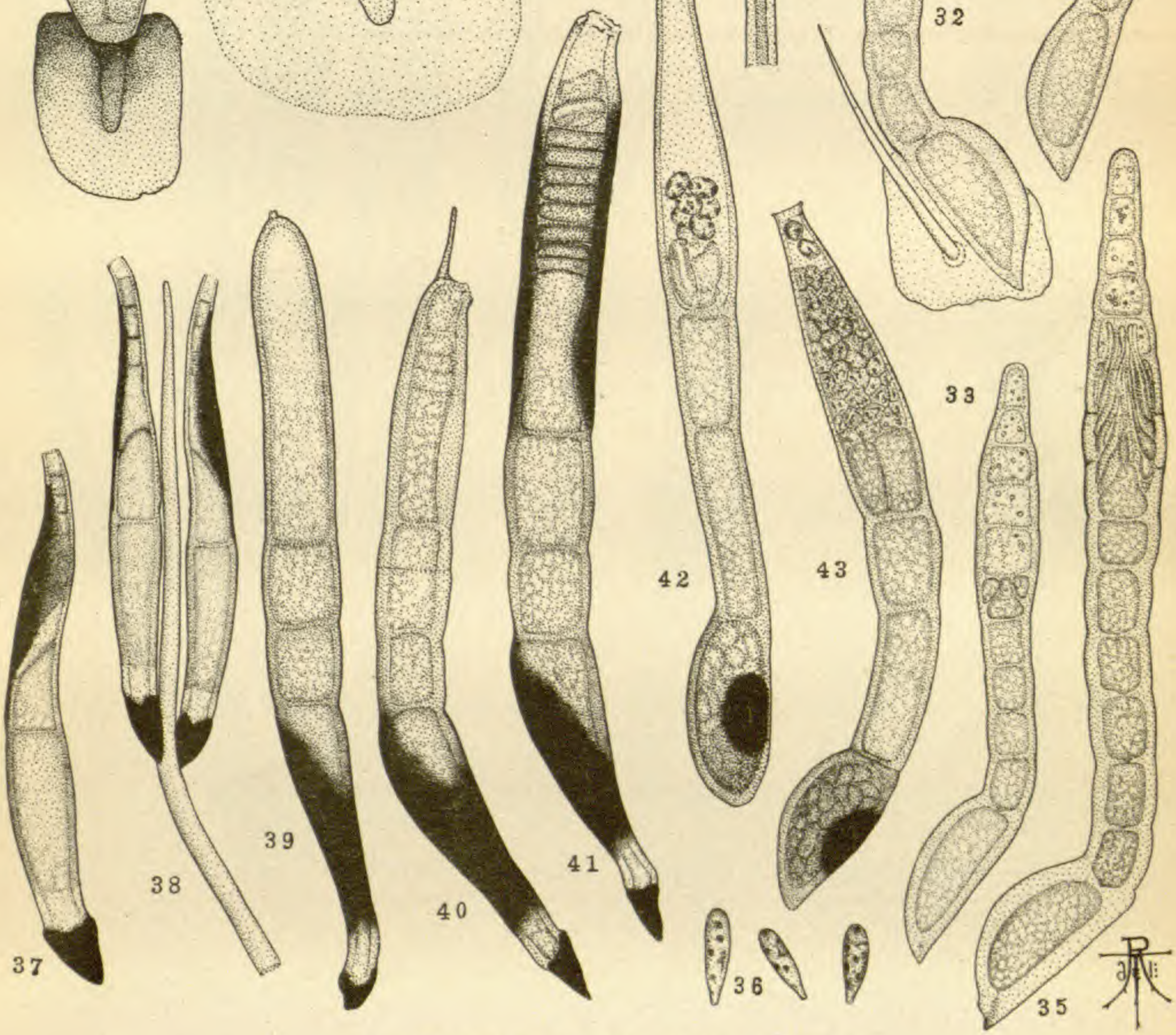

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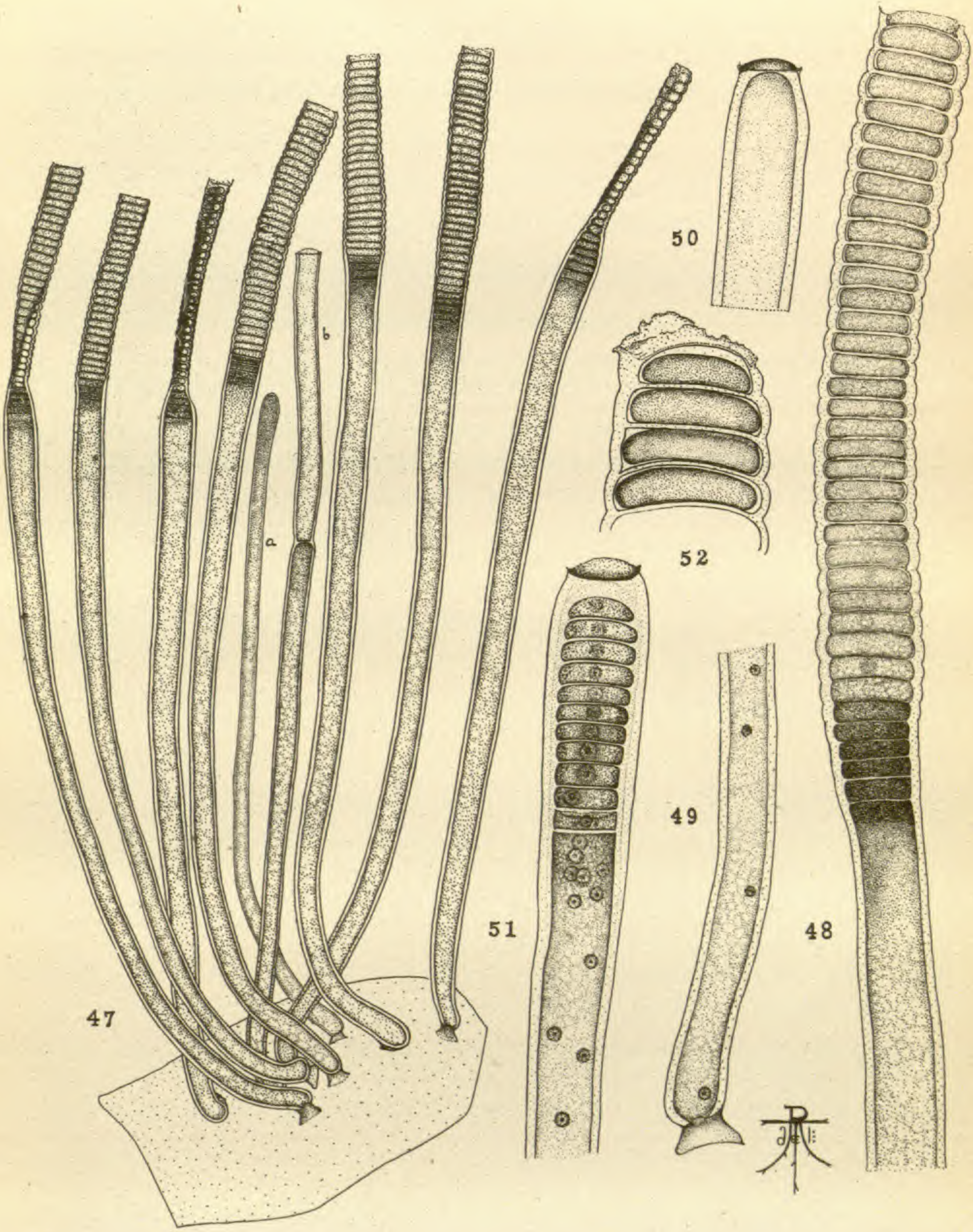

THAXTER on FUNGUS-PARASITES

Endosporella Diopsidis Thaxter

FIG. 39.—Young individual in which sporulation has not begun, although terminal spine has been broken off; 10, 4.

FIG. 40.—Older individual, spine persistent and sporulation beginning; 10, 4.

FIG. 41.—Mature individual, distally perforate; 10, 4.

Laboulbeniopsis Termitarius Thaxter

FIG. 42.—Mature individuals, basal cells of sporogonium disorganized, a few residual somewhat swollen spores remaining; 10, 4.

FIG. 43.—Mature individual, spores beginning to escape; 10, 4.

Amphoromorpha Blattina Thaxter

FIG. 44.—Front view of mature individual removed *in toto* from antenna of host, showing unevenly spreading secretion over simple rhizoidal foot; 10, 4.

Amphoromorpha entomophila Thaxter

FIG. 45.—Base of mature individual completely filled with spores and with no large basal cell, showing rhizoidal foot and secretion as in preceding figure; 10, 4.

Amphoromorpha sp.

FIG. 46.—Two individuals growing on antennal hair; foot seen laterally, but visible through transparent secretion; 10, 4.

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

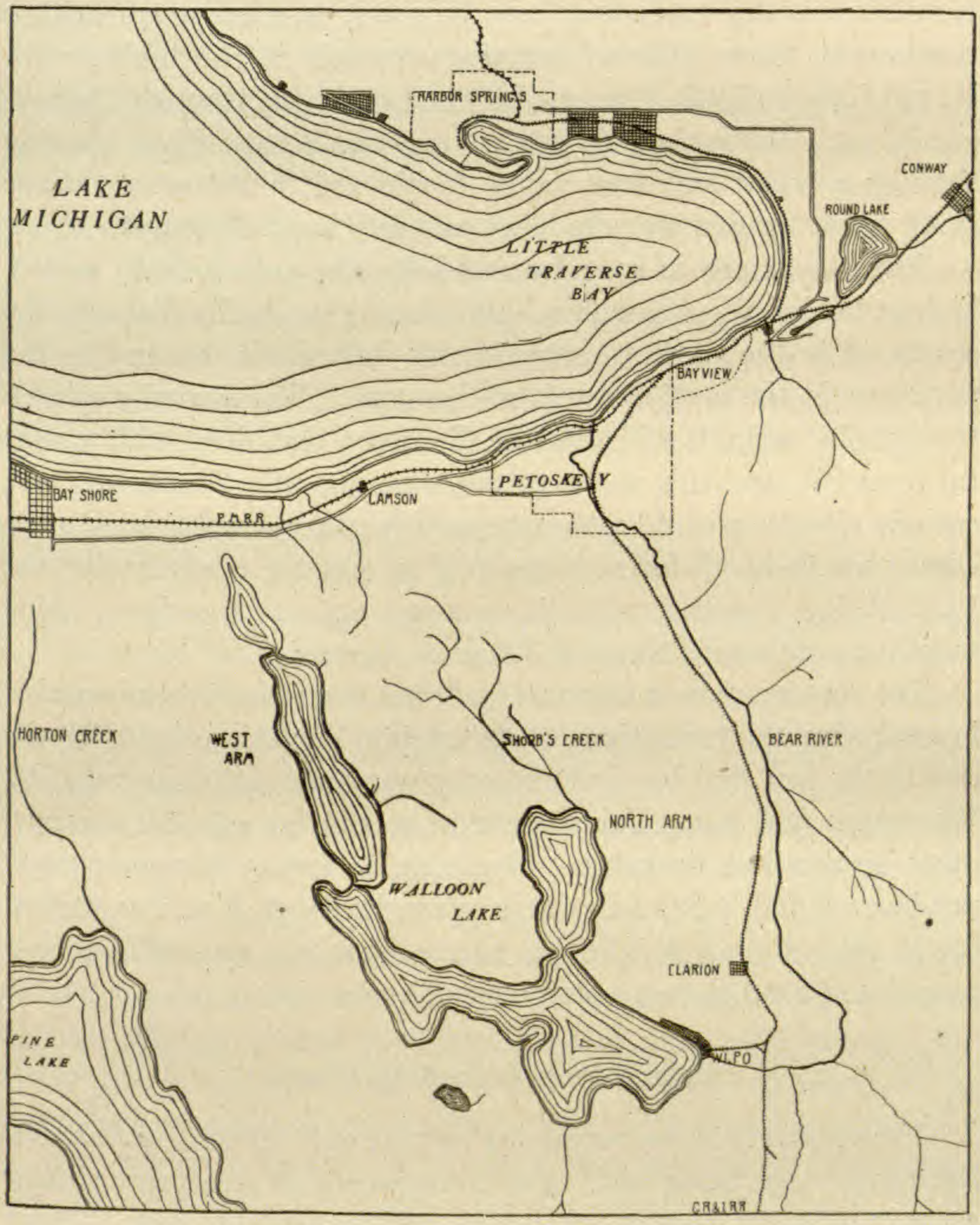

FIG. 1

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.

Mosses are not common on vertical trunks. Ferns are not seen as epiphytes in this region, though not from lack of either individuals or species. Both may be found growing on rotting stubs (not hemlock).

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.

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OAK PARK, ILL.

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FIELD AND LABORATORY STUDIES OF VERBENA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 257

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.

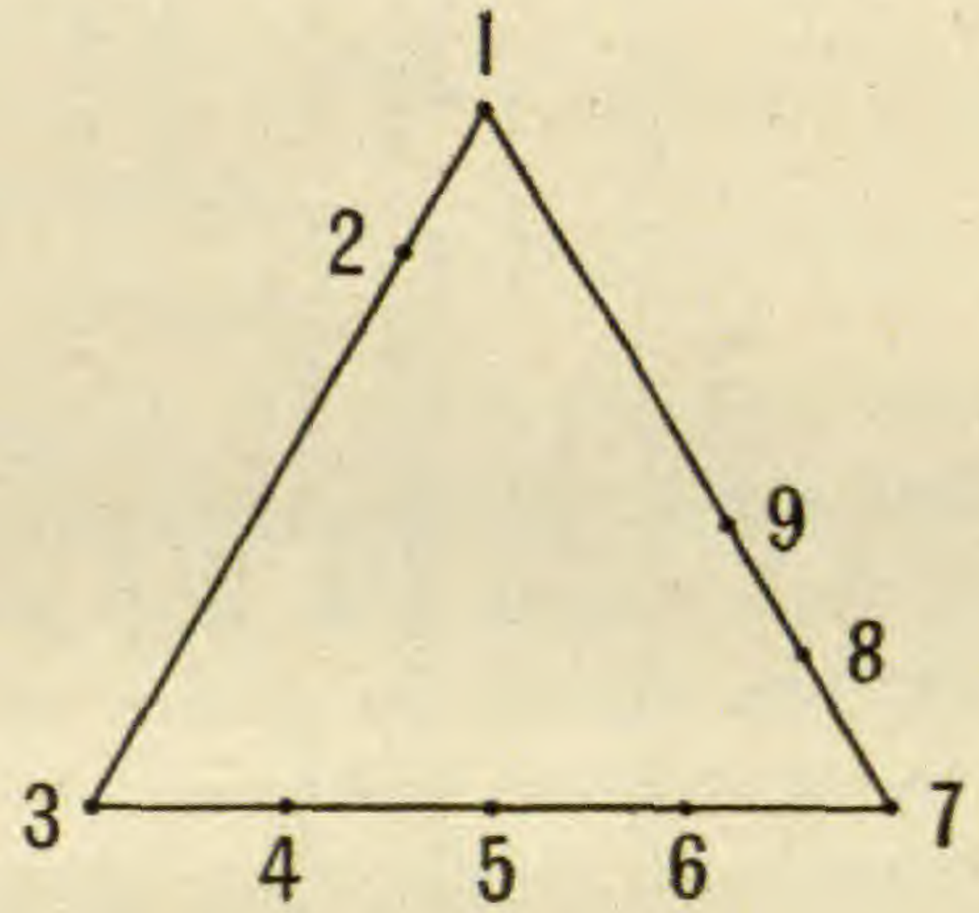


FIG. 10.—Diagrammatic representation of morphological relationship between originals and intermediates.

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

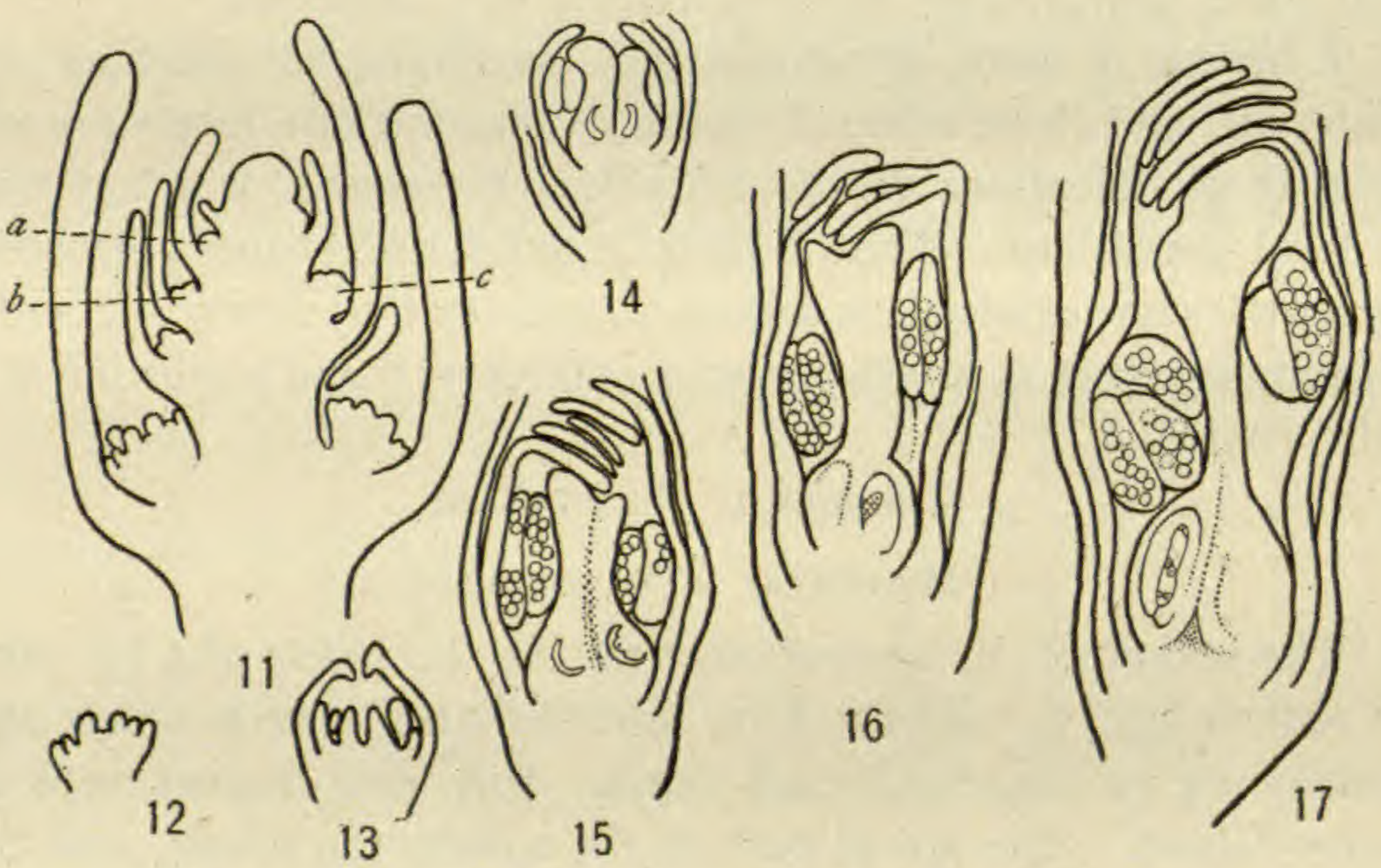
Material and methods

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 sub-epidermal 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.

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.

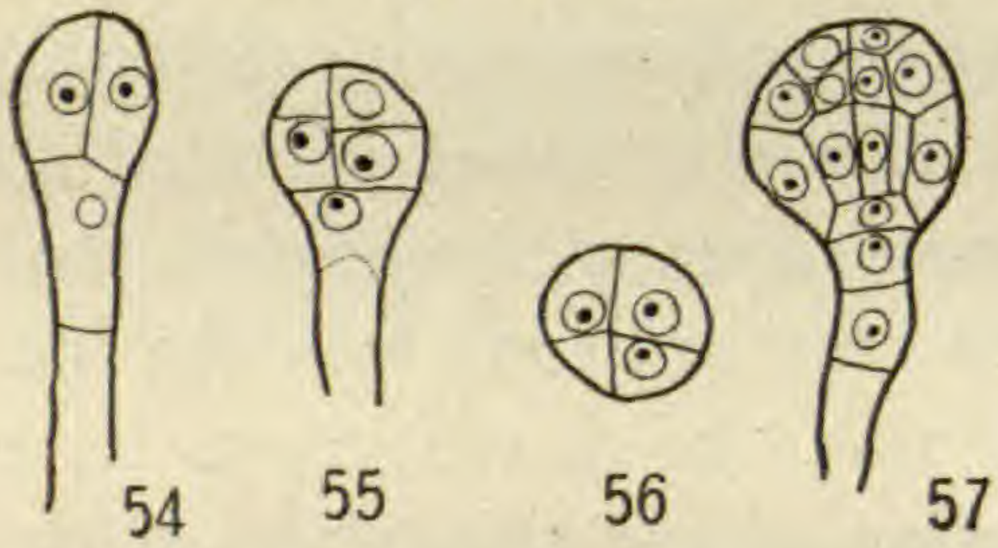
A large amount of starch is present in the embryo sac, as was also observed by GUIGNARD (7) (*Cestrum*), D'HUBERT (5) (Cactaceae), WEBB (18) (*Astilbe*), and LLOYD (12) (*Galicium*). This is observable not only a little before fertilization, but more especially after fertilization has occurred (figs. 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.

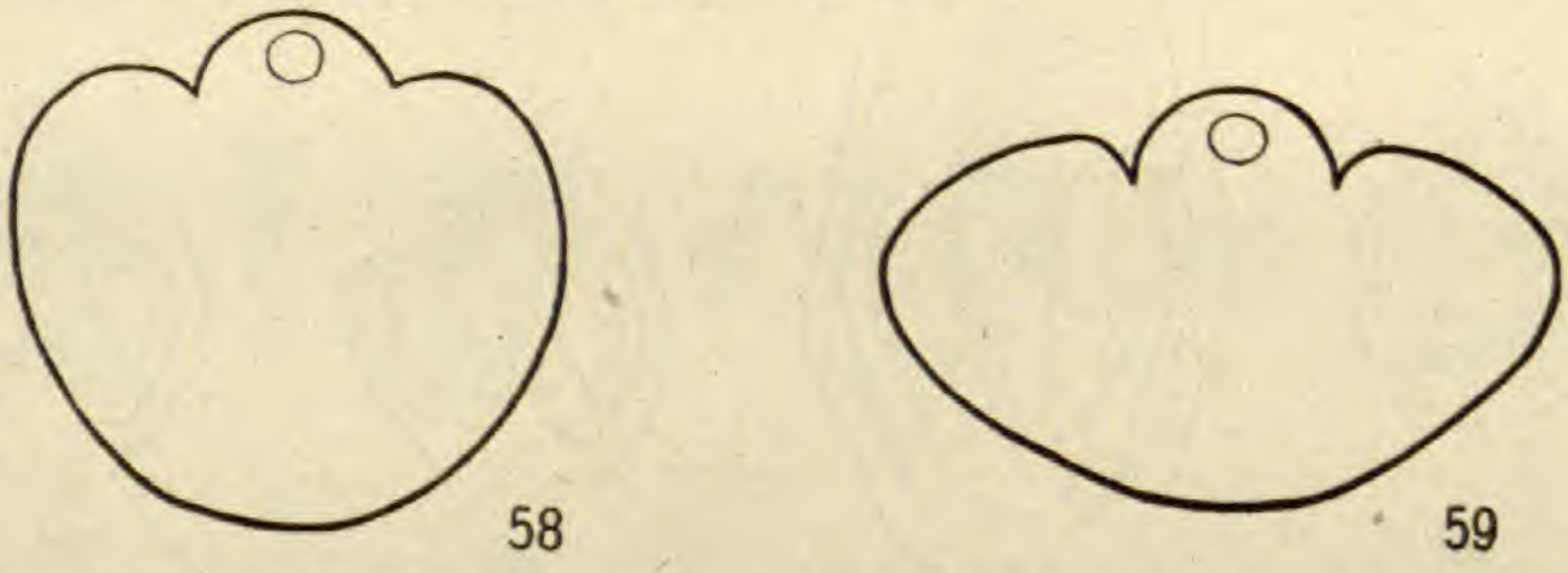
The dermatogen, periblem, and plerome layers are next differentiated in the embryo (fig. 57), which now occupies the end of a long suspensor. The appearance is identical with that of *Capsella*.

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.

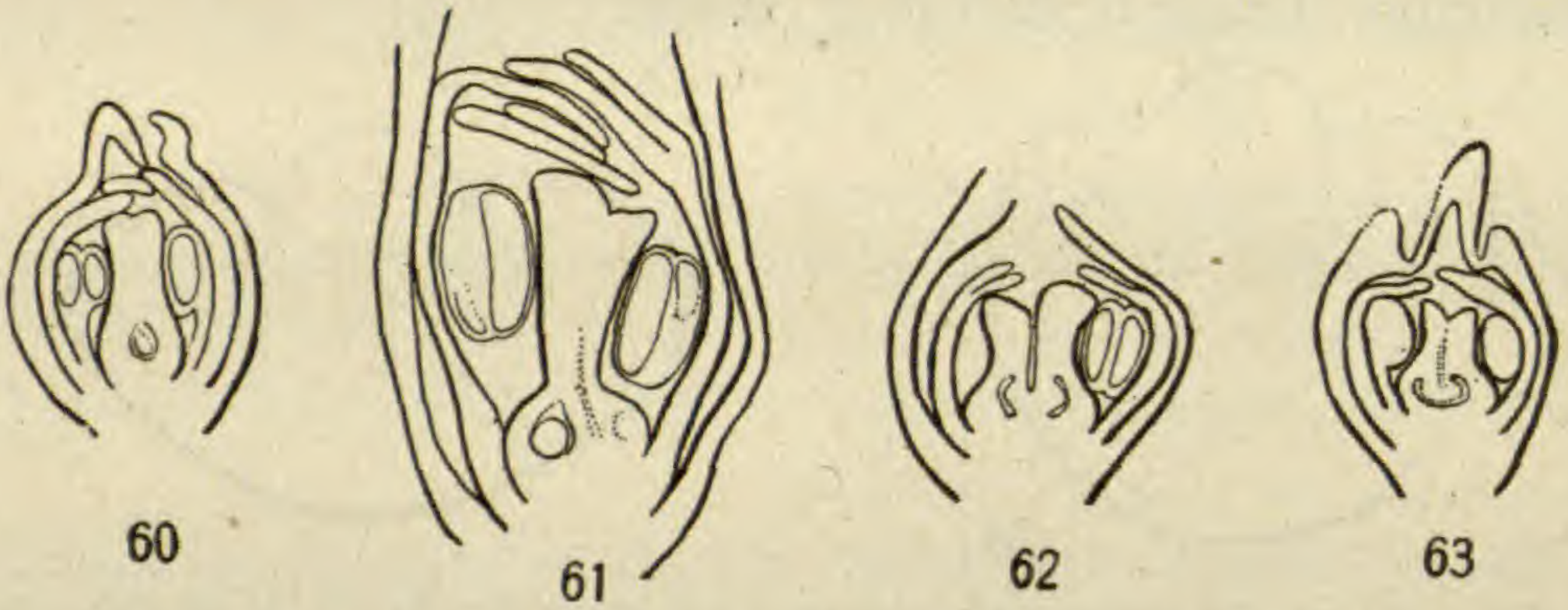
TABLE II

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



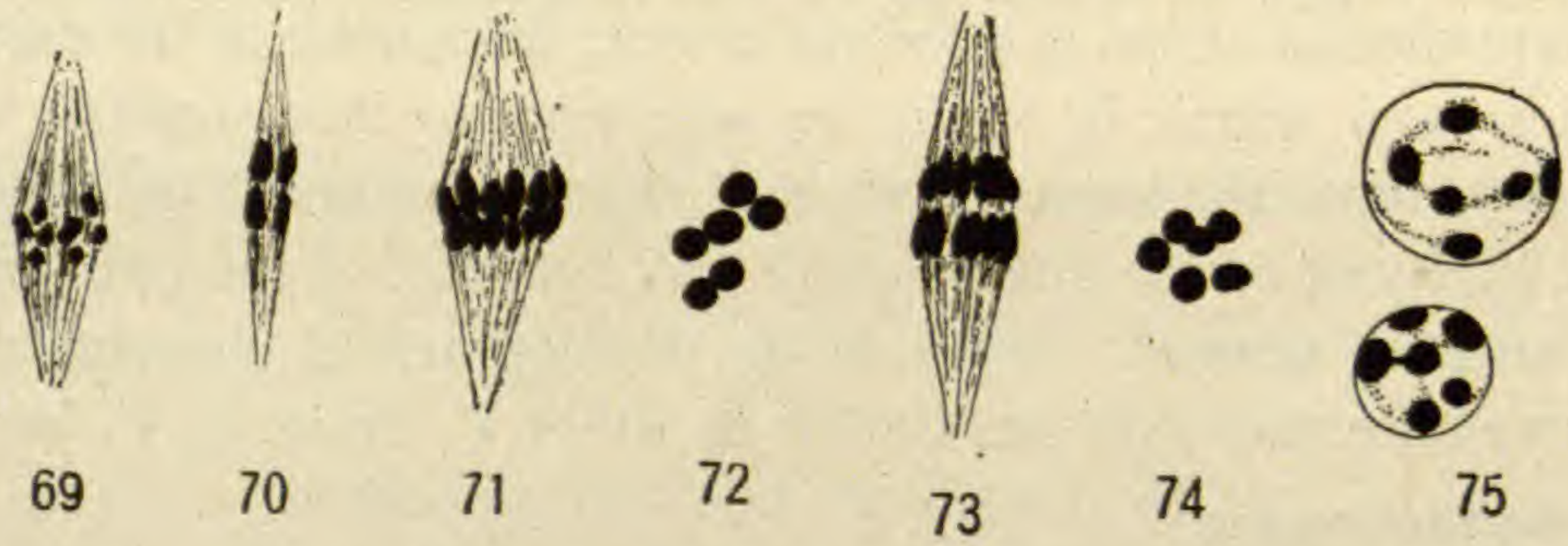
FIGS. 60–63.—Comparison of florets at time of first mitosis in pollen mother cells: fig. 60, *V. angustifolia;* fig. 61, *V. stricta;* fig. 62, intermediate form between *V. stricta* and *V. hastata;* fig. 63, *V. hastata;* ×35.

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*.

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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.

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.

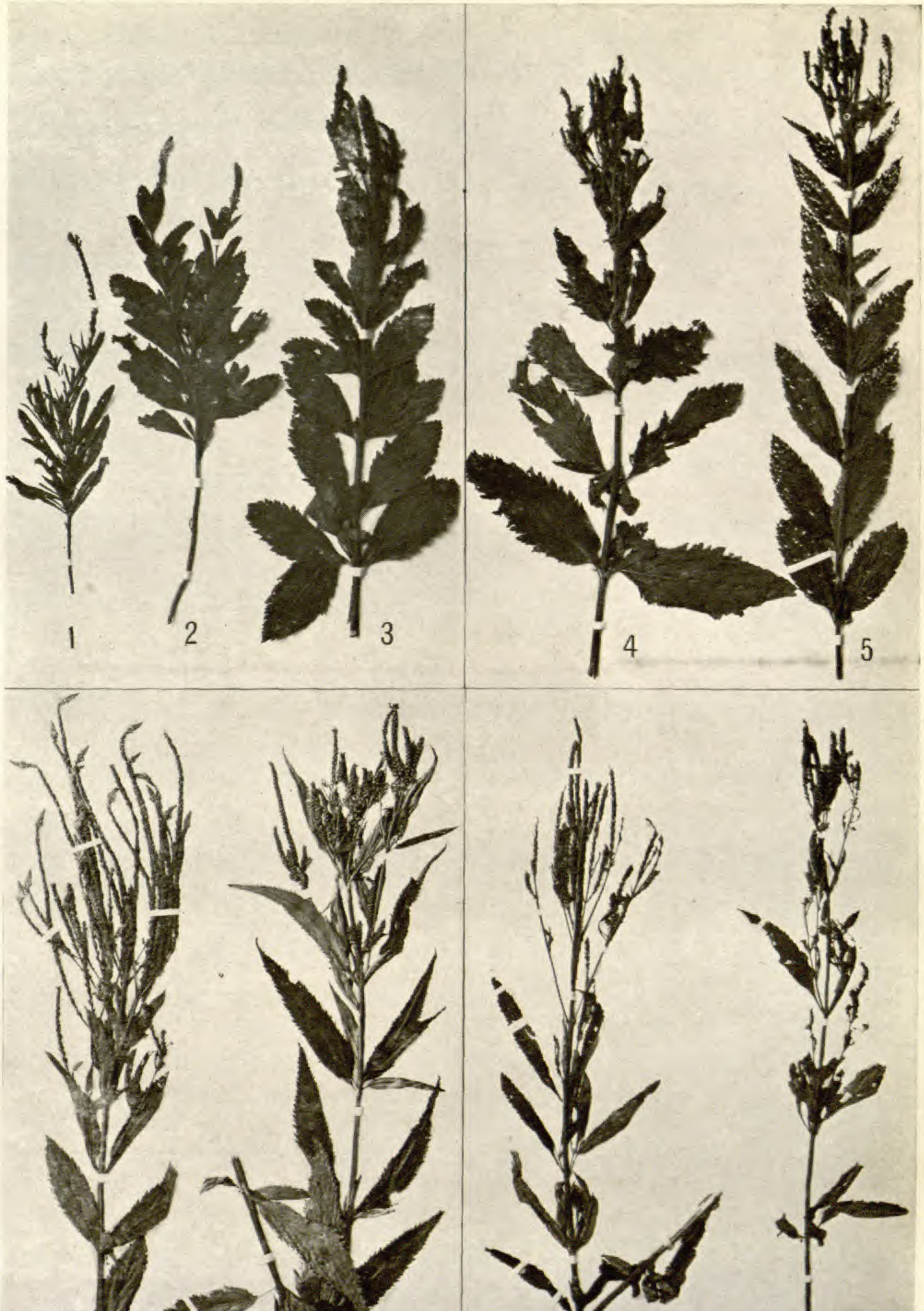
FIG. 36.—Tetrad formation; cytoplasm of mother cell not concerned in wall formation.

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.

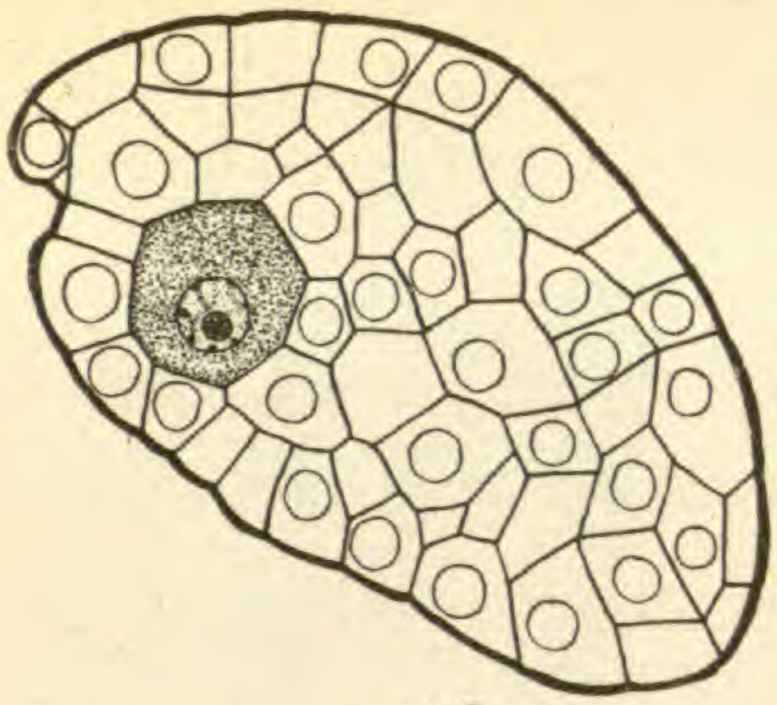
FIG. 42.—Pollen tube just thrusting itself through nucellar cap.

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.



KANDA on VERBENA



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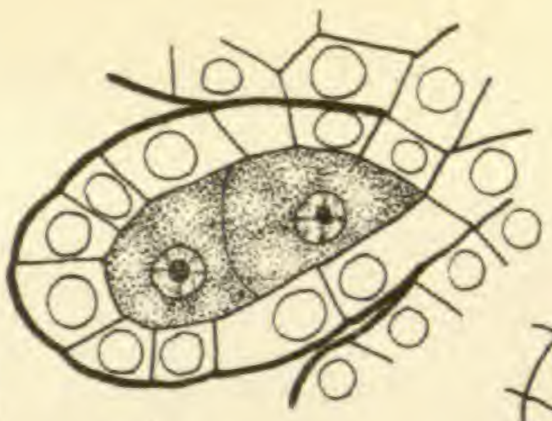

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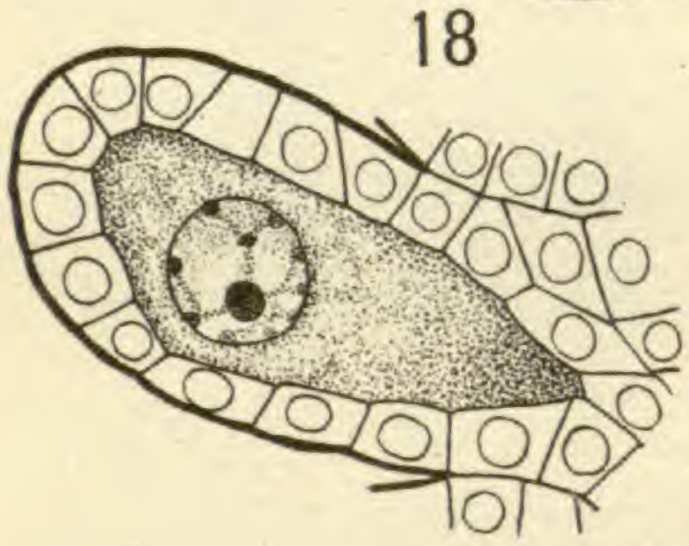

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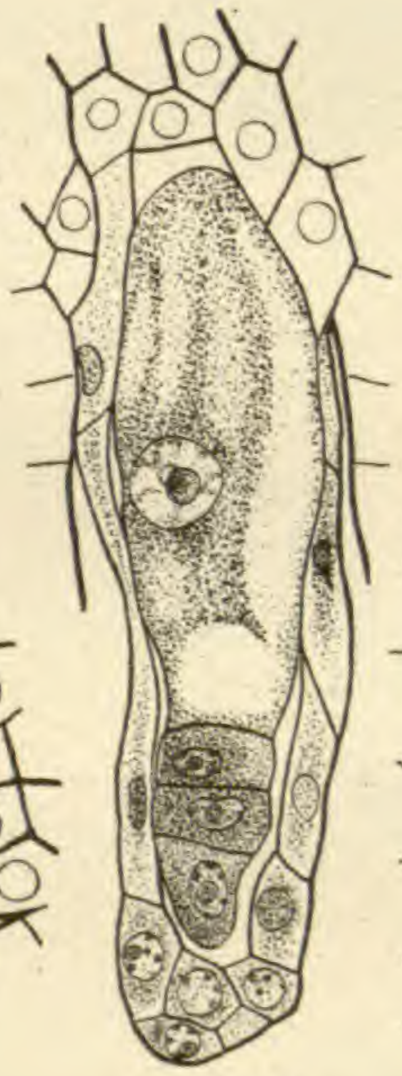

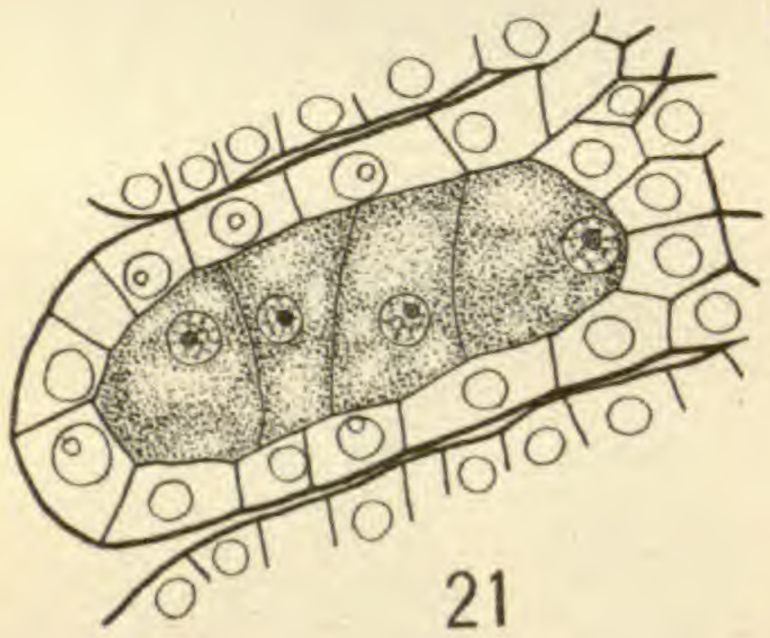

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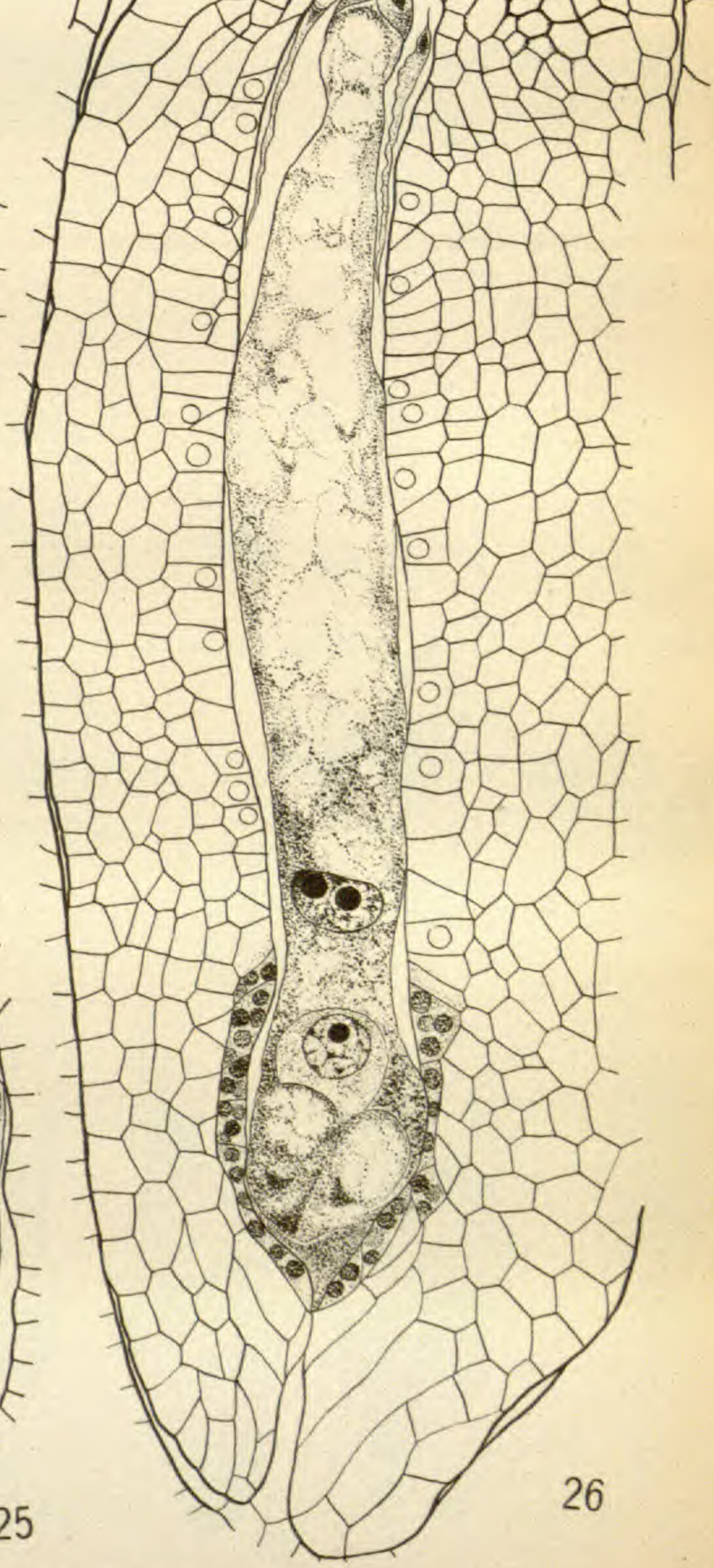

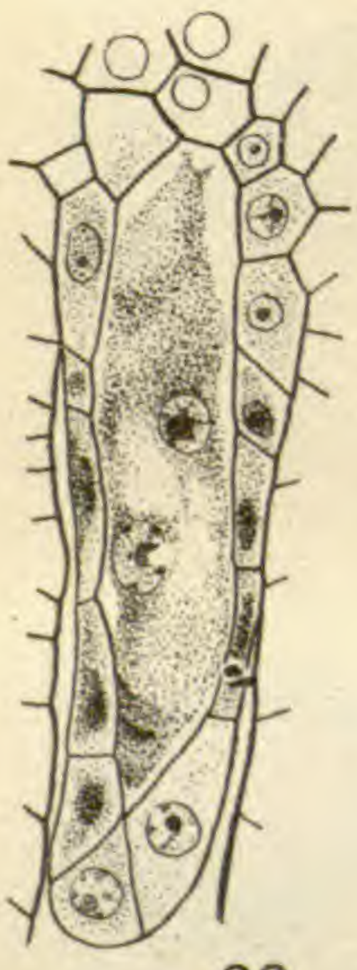

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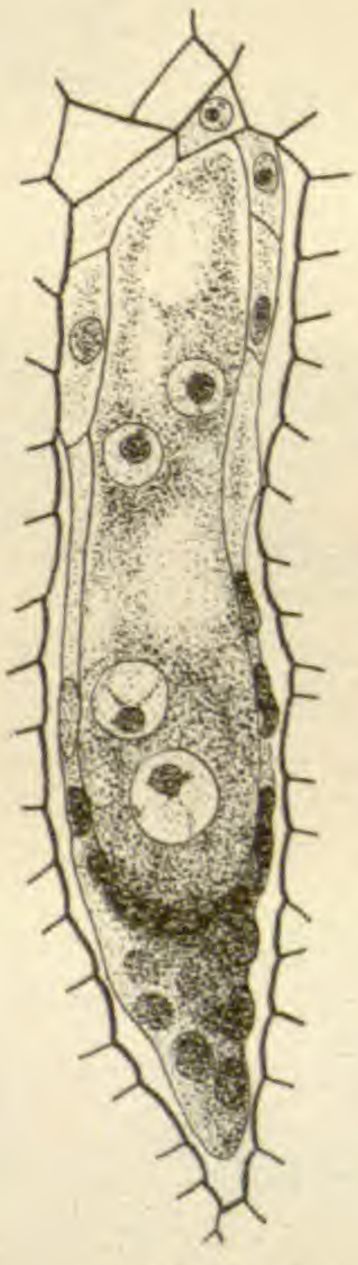

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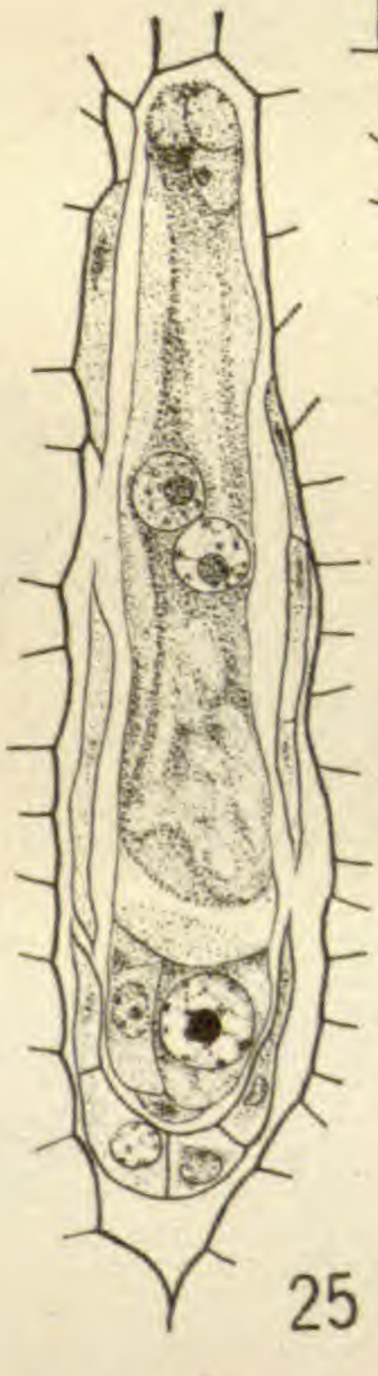

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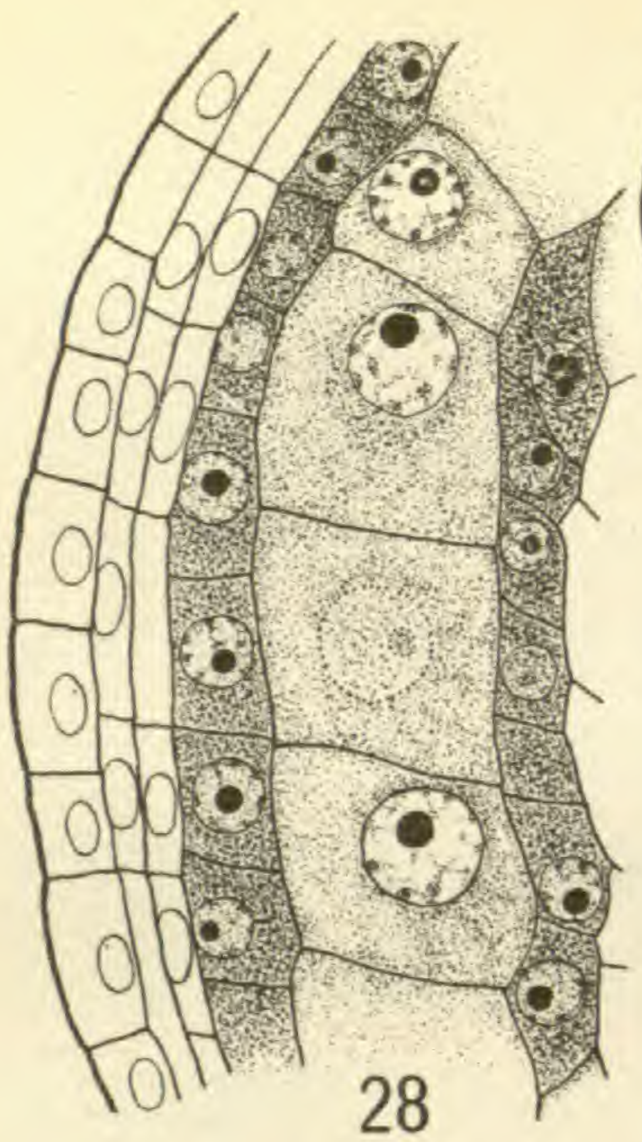
KANDA on VERBENA



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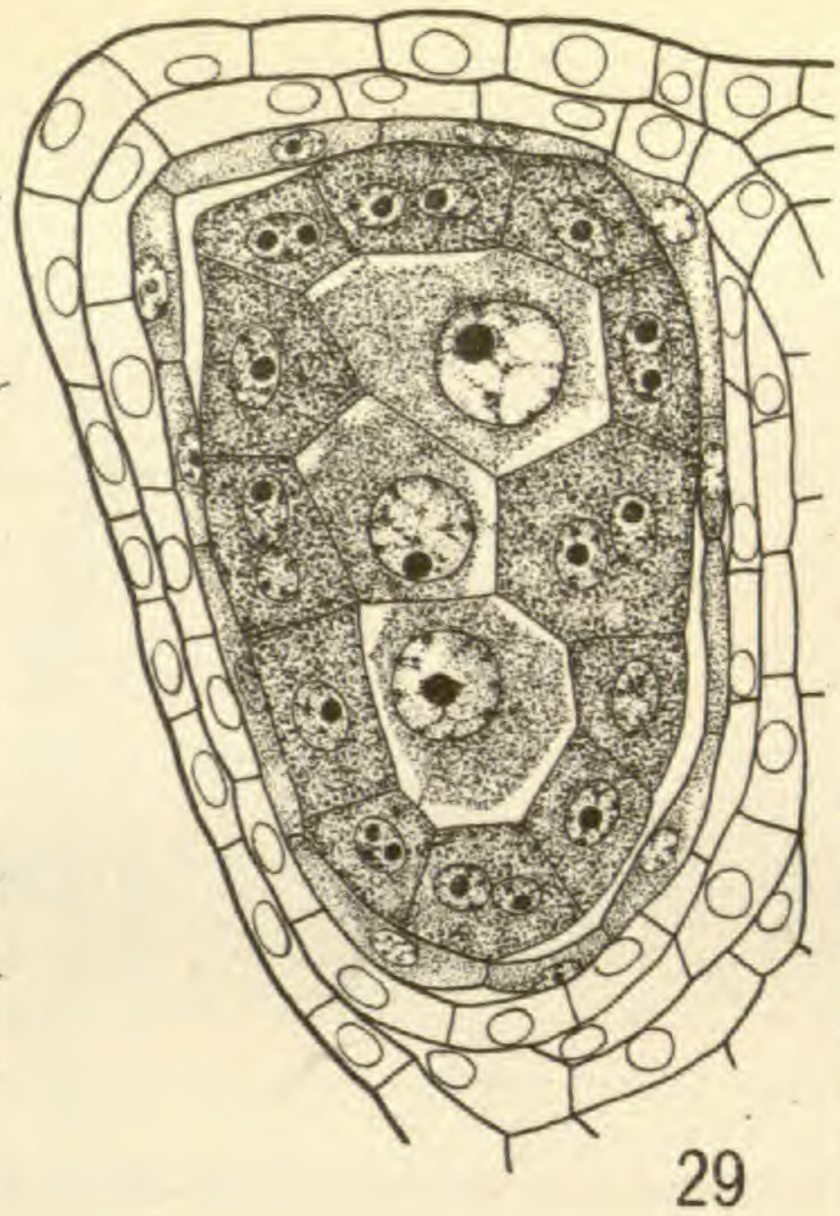

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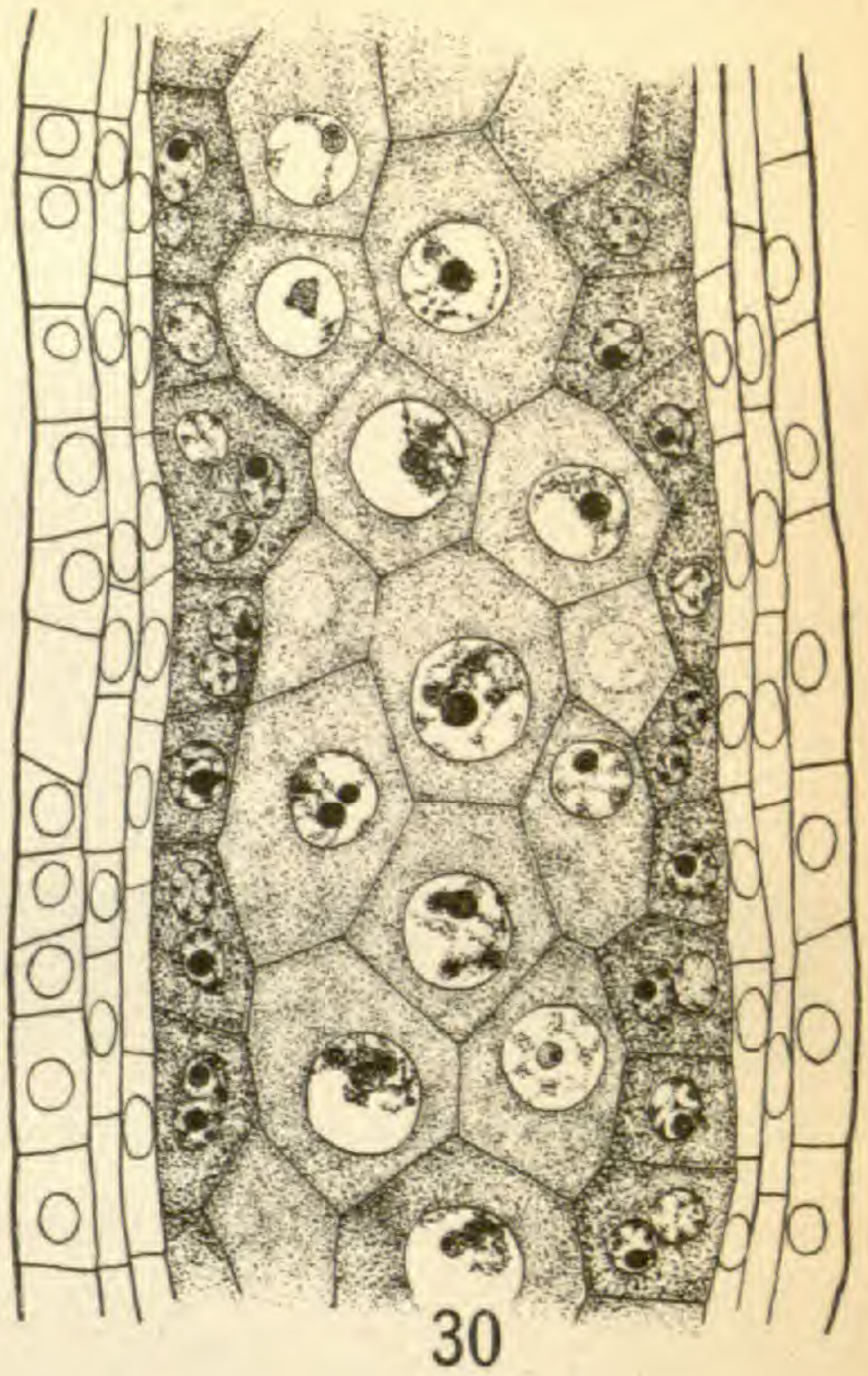

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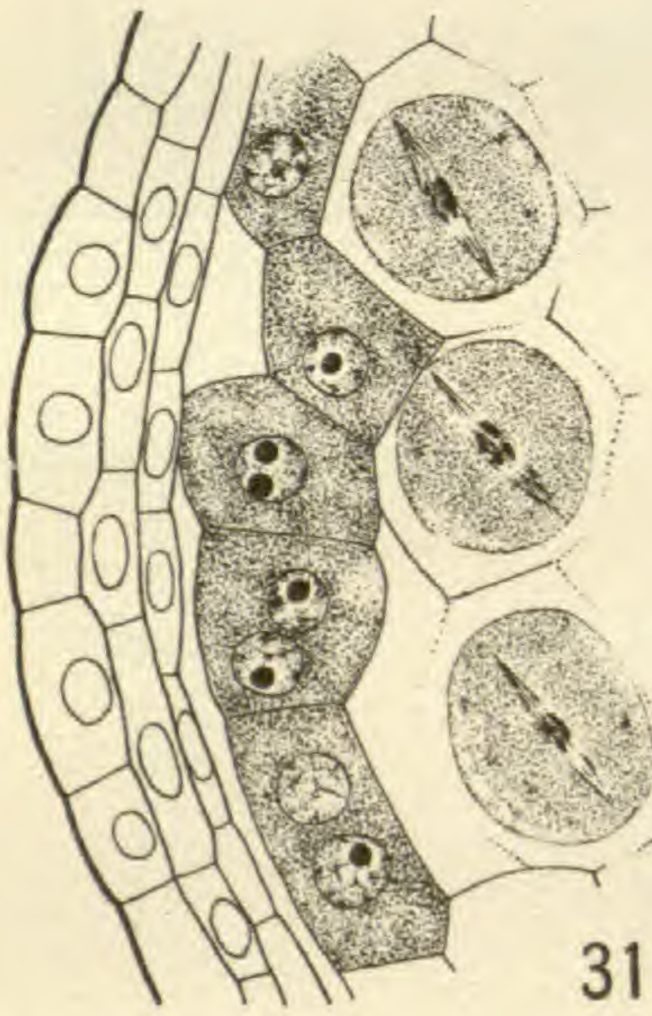

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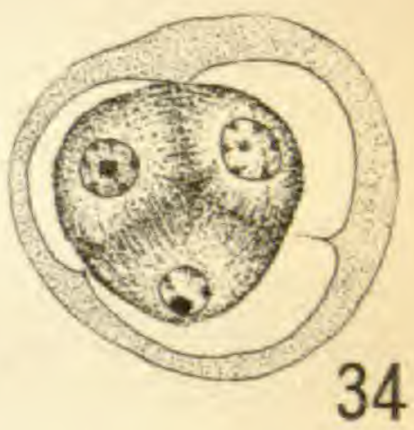

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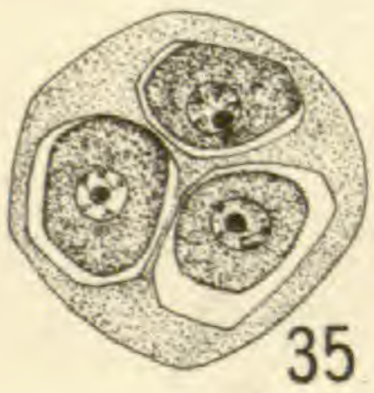

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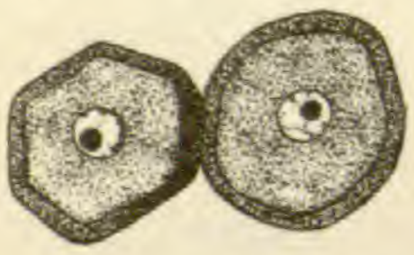

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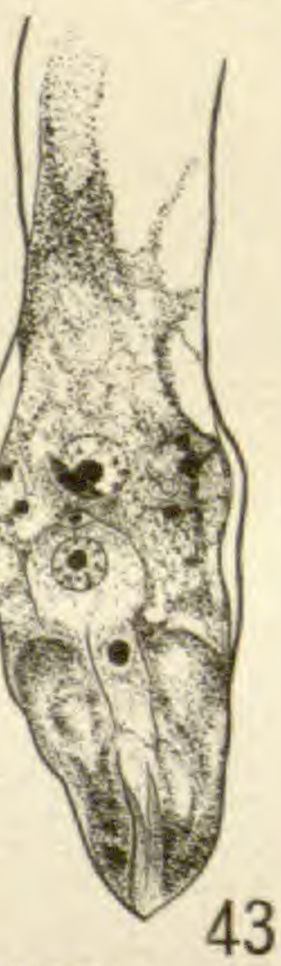

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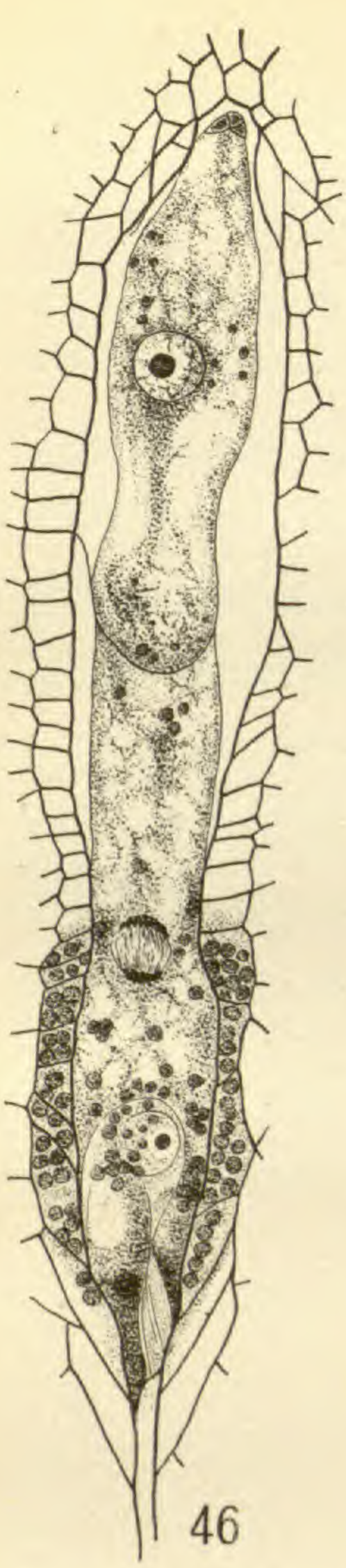
KANDA on VERBENA



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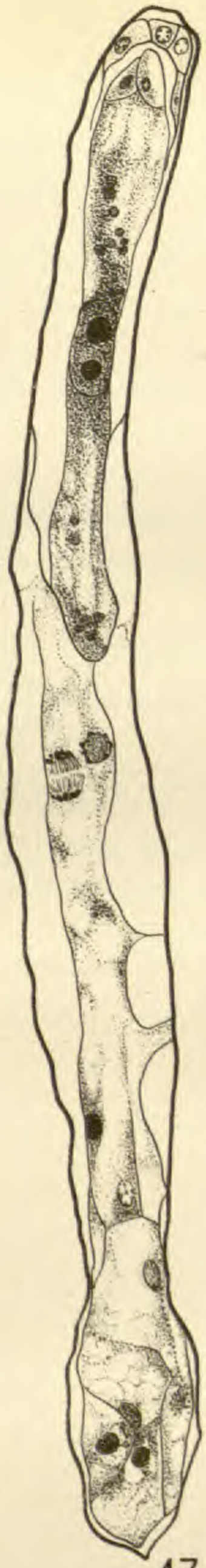

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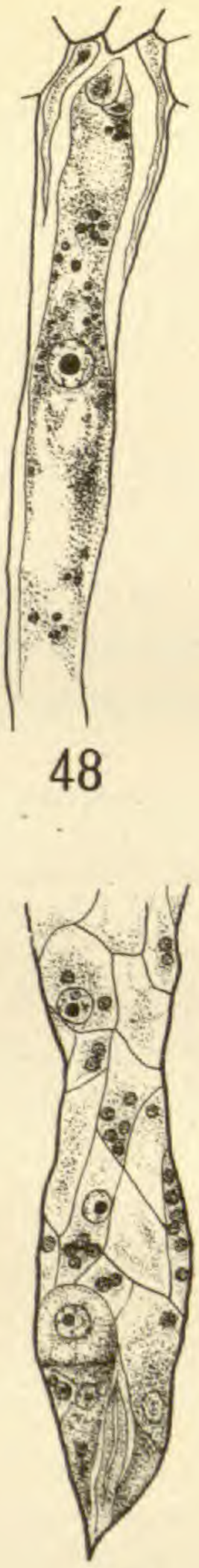

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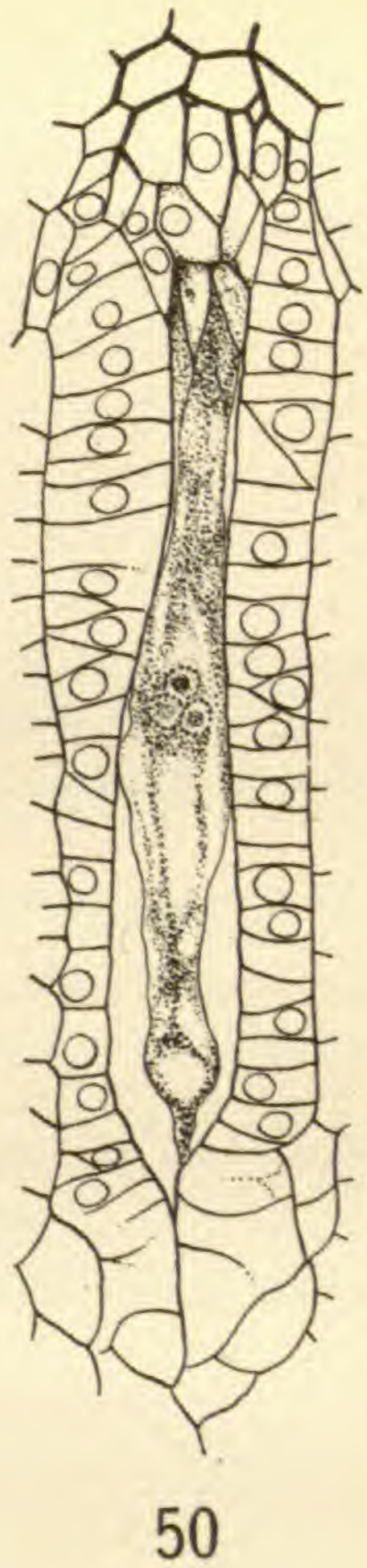

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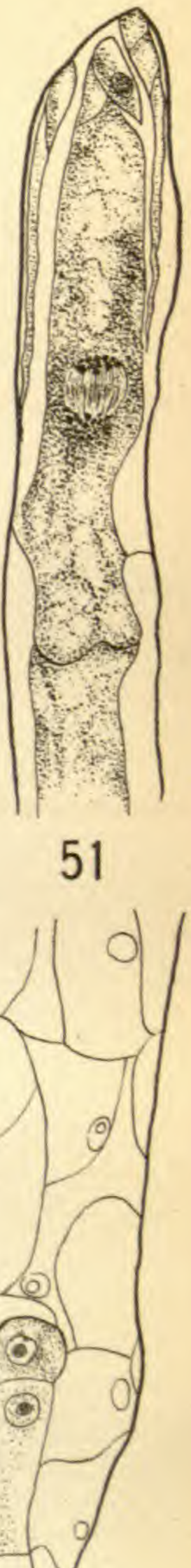

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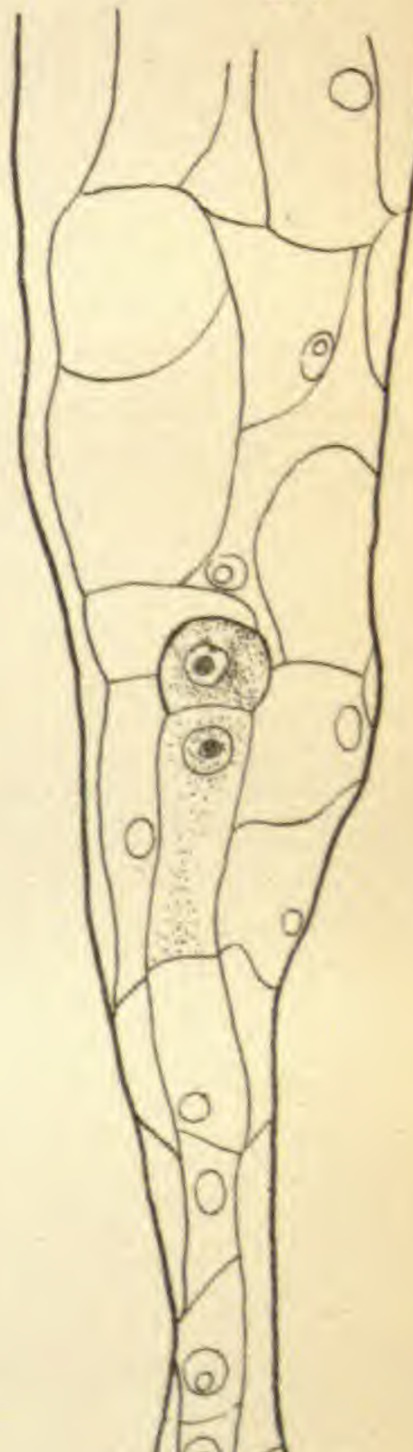

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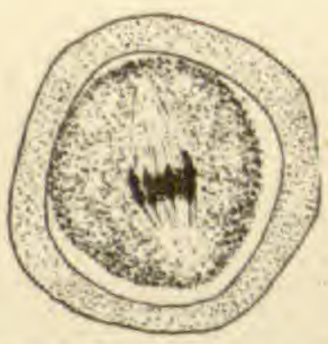

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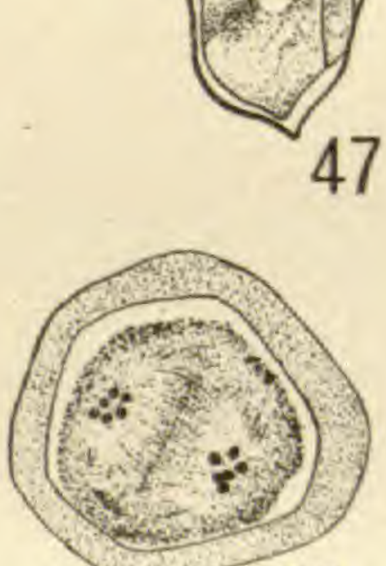

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KANDA on VERBENA

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

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 animal 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 cen t

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	1.5–3.7
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	2–5
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.

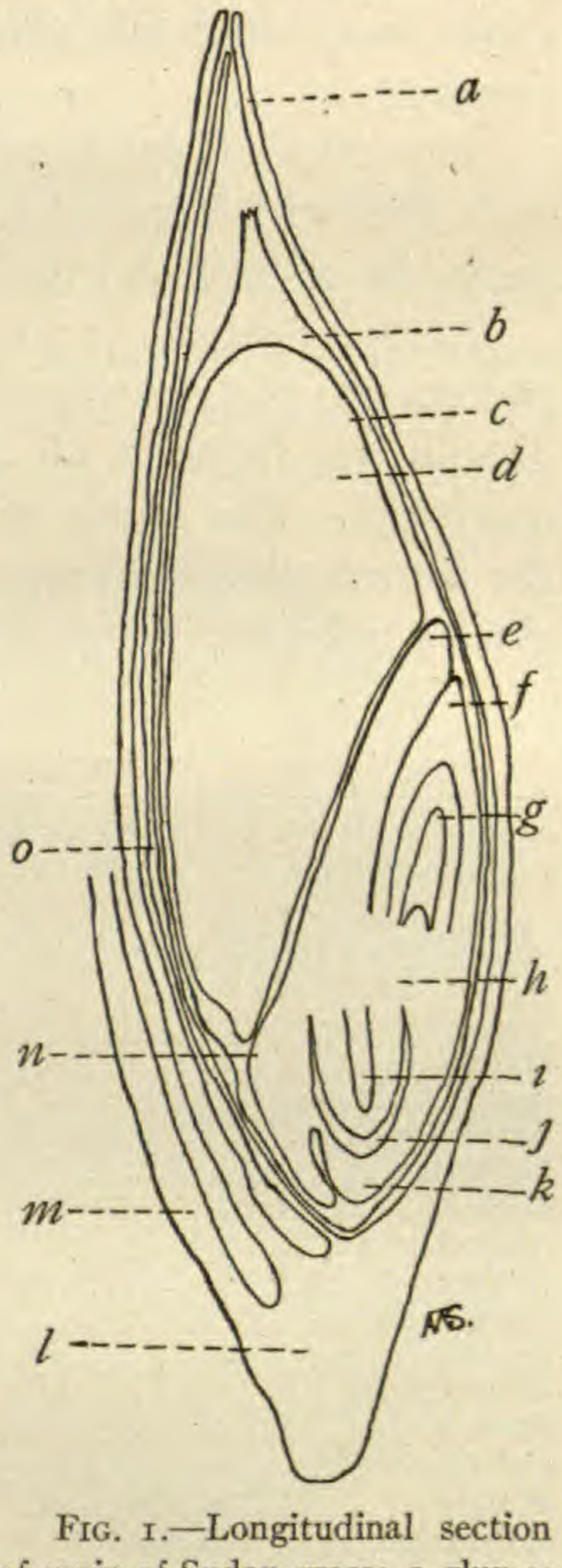

FIG. 1.—Longitudinal section of grain of Sudan grass: *a*, glume; *b*, pericarp; *c*, aleurone layer; *d*, endosperm; *e*, scutellum; *f*, coleoptile; *g*, plumule; *h*, embryo node; *i*, radicle; *j*, root cap; *k*, coleorhiza; *l*, pedicel; *m*, basal seta; *n*, glandular layer of scutellum; *o*, lodicule.

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.

BRIEFER ARTICLES

WILLIAM GILSON FARLOW

(WITH PORTRAIT)

With Dr. FARLOW, whose death occurred on June 3, 1919, after a short illness, there passes not only a unique personality, but one whose preeminence in his special field was such that to no one else could the title of cryptogamic botanist, in the broader sense, be so justly applied. Apart from his extensive familiarity with other branches of botany, it is doubtful if anyone has ever approached him in his knowledge of the non-vascular plants as a whole, a knowledge so comprehensive as well as so detailed, that in matters relating to most of the larger groups his opinion was rightly regarded as that of an expert.

Gifted with an extraordinarily retentive memory, exceptional ability, keen discernment, and sound judgment; appreciating the necessity for a wide and thorough training for his work; possessing, also, sufficient means with which to avail himself of opportunities, many of which were such as come only to the pioneer, he was able to accumulate books, collections, and other material needs for the execution of his purposes. His equipment thus included intellectual and material factors which combined to make him one of the foremost figures in the botanical world.

Dr. FARLOW'S interest in botany had already developed during his undergraduate days at Harvard, and his natural fondness for the subject was fostered and developed by his contact with ASA GRAY, by whose

advice, after graduation, he studied medicine in preparation for a scientific career. Receiving his Doctor's degree in 1870, he became GRAY'S assistant, and had the privilege of teaching and studying with him for two years. Although, during this association, he gained a comprehensive knowledge of the vascular plants, his preference for the non-vascular types, and especially the algae, was already apparent, since it is with the latter that his first two papers, "Cuban seaweeds" (1871) and "List of the seaweeds or marine algae of the south coast of New England" (1871–1872), are concerned.

GRAY'S interests, being primarily systematic, were naturally impressed on Dr. FARLOW, and the former evidently contemplated the conversion of his pupil into a collaborator who might in a measure do for the lower cryptogams what he had himself done for the flowering plants, even to the point of preparing a manual. Although no portion of this program was carried out, the preparation of a textbook of cryptogamic botany was in Dr. FARLOW'S mind more or less constantly, until the idea was finally abandoned in the early nineties. It was partly with this in view that he was advised by GRAY, after serving two years as his assistant, to visit Europe, come in personal relations with European botanists, acquire a knowledge of their methods of working and of teaching, and above all to learn as much as possible of the lower forms, especially the fungi and lichens. He therefore sailed for Liverpool in June 1872, and went first to Scandinavia, where he saw, among others, the elder FRIES, as well as ARESCHOUG and AGARDH and their herbaria. He continued his journey as far as St. Petersburg, where he desired to see the algae in the Ruprecht Herbarium. Although he also traveled in Germany, Switzerland, France, Italy, and England, meeting many well known botanists, he passed most of his time at Strassburg in DEBARY'S laboratory, spending also some weeks in an intensive study of the lichens with Dr. J. MULLER at Geneva, and of the algae with BORNET and THURET at Antibes. DEBARY was then professor of botany and regent of the German University, which had replaced the French Académie after the close of the Franco-Prussian War, and was reputed to know more about the fungi, their morphology and development, than anyone else in the world. Dr. FARLOW was thus able to fill this, the most serious gap in his equipment, and to acquire, among other things, a good foundation in general plant anatomy. Here he came in contact with SCHIMPER, then an old man and the most distinguished member of the scientific faculty, Graf SOLMS, recently appointed ausserordentlich professor, and various students attracted by DEBARY'S courses: STAHL,

ROSTAFINSKI, SOROKIN, GILKINET, LINDSTEDT, and others. He was strongly influenced by the personality of DEBARY himself, his wide knowledge, ability, earnestness, and high ideals of care and accuracy in scientific work. The training which he thus acquired served as a fitting complement to that which he received from ASA GRAY, the impress of whose systematic predilections was thus tempered by DEBARY'S very different point of view. Work of a taxonomic or even of a general nature was not encouraged in the latter's laboratory, and he was regarded by Dr. FARLOW as somewhat narrow in his conception of the scope and extent of the preparation desirable in the preliminary training of a botanist. He was not himself, however, restricted to a special topic until more than a year after he entered the laboratory, when DEBARY, having observed the vegetative development of a fern sporophyte from the prothallus, turned the subject over to him for investigation. The resultant paper, on "An asexual growth from the prothallus of *Pteris cretica*," published in the *Botanische Zeitung* and elsewhere, attracted wide attention and interest, and, although it was at first attacked from all sides, rendered his name familiar to botanists everywhere.

His reputation was thus well established when he returned to America in the summer of 1874, and was appointed to an assistant professorship at Harvard, the first special provision in this country for instruction in cryptogamic botany. For some years he was stationed at the Bussey Institution, where his work dealt largely with the economic aspects of mycology, and where he may be said to have laid the foundations of American phytopathology. During this period of 5 years his published papers on fungi were largely devoted to destructive parasites, such as the black knot, grape mildew, onion smut, etc., although he did not neglect the marine algae, and published several articles on the algal impurities of water supplies.

In 1879 he was transferred to Cambridge as professor of cryptogamic botany, a position which he continued to occupy until his death, after a service on the Harvard faculty of 45 years. He was thus able to devote himself to the Farlow Herbarium, the nucleus of which was the well known Curtis Herbarium, purchased during his absence in Europe, and of his unrivaled library of books, papers, and journals relating to cryptogamic botany; the development of instruction in different branches of the subject, as well as of productive investigation on his own part and that of his students.

In 1883 he instituted the numbered series of "Contributions from the Cryptogamic Laboratory of Harvard University," which, up to the

time when he retired from active teaching in 1896, included the titles of some 40 papers, which, with the exception of the four first numbers written by himself, represent original work accomplished by his students. Among the latter were included B. D. HALSTED, WILLIAM TRELEASE, J. E. HUMPHREYS, W. A. SETCHELL, K. MIYABE, H. M. RICHARDS, and other well known names of American botanists. His own publications during this period were numerous, and included, for example, "Monograph of the Gymnosporangia," "Marine algae of New England," "Host index of fungi," etc. It is greatly to be regretted that his *magnum opus*, on selected species of fleshy fungi, for which an edition of very beautiful plates was printed long before his death, has been left uncompleted.

Although he continued a member of the Harvard faculty until his death, he withdrew from teaching in the year just mentioned, giving attention occasionally to advanced students in whose work he felt a special interest, devoting himself chiefly to the care and increase of the herbarium and of his library, as well as to the supervision of the extensive "Bibliographical index of American fungi," the first part of which, prepared in collaboration with A. B. SEYMOUR, was published by the Carnegie Institution in 1905. At the same time he kept up his botanical reading, about which he was hyperconscientious, and which was varied and extensive, being by no means limited to matters relating to cryptogams alone; while he also carried on a voluminous correspondence, sparing neither time nor trouble to assist those in search of advice or information as to identities, synonymy, or literature.

Throughout his life Dr. FARLOW was an indefatigable collector, and his activity of body and keen eyesight, which were little impaired by age, combined with his long experience and wide and exact knowledge, enabled him to detect a host of new, rare, or interesting forms. His annoyance at encountering unrecognizable, and in numberless instances undoubtedly new, forms, was often very amusing. He had so little patience with species makers that he himself described but a very small percentage of the novelties that came in his way. Of those who make a profession of this type of botanical activity he once said to his class, "If a difference can be *imagined*, it is a new species; if one can be *seen*, it is a new genus." A number of new genera and species were none the less named in his honor, of which he laughingly asserted that "they were almost all bad."

Dr. FARLOW's attainments, his rare ability and learning, commanded the respect of all who came in contact with him, and were given recogni-

tion by the bestowal of honorary degrees (LL.D. by Harvard, Wisconsin, and Glasgow, and Ph.D. by Upsala), as well as by his election to membership in the National and Paris Academies of Science, the American Philosophical Society, the American Academy of Arts and Sciences, the Linnaean Society of London, and various other scientific bodies in this country and abroad. His good judgment, keen sense of humor, originality, and faculty for interesting presentation never failed to render any public deliverance of his a memorable event.

There are few that have been brought into close relations with him as students, or in scientific work, whose standards and ideals he did not fundamentally influence; while those who had experienced his unfailing kindness, thoughtfulness, and sympathetic interest not only regarded him with the honor and respect due to his character and attainments, but with a personal feeling of obligation and affection.—ROLAND THAXTER, *Harvard University*.

CURRENT LITERATURE

NOTES FOR STUDENTS

Mitosis in Osmunda.—Cytologists are familiar with the two outstanding views, associated respectively with the names of GRÉGOIRE and FARMER, regarding the method of chromosome reduction. According to the first view the doubleness of the spirem of the early heterotypic prophase, unlike that of the somatic prophase, is due to a lateral conjugation of threads representing entire chromosomes to form bivalents which are separated at the heterotypic mitosis, a new split functioning in the homotypic. According to the second view the doubleness is due to a split as in somatic mitosis; bivalents are formed by a conjugation of segments of this double spirem which separate in the first mitosis, while the original split functions in the second. A very complete statement of this latter interpretation has been given by Miss DIGBY[1] in a new account of mitosis in *Osmunda*.

In all the archesporial divisions, including the last, the chromosomes undergo a longitudinal splitting during early telophase. The homogeneous daughter threads become beaded as the split between them widens, and with many small connecting strands eventually form a faint resting reticulum which bears many small granules, and in which the limits of the individual chromosomes are indistinguishable. Most of the chromatin is collected in three or more nucleoli. In the succeeding prophase the reticulum resolves itself into a number of thin beaded linin threads; these run in parallel pairs and are regarded as the two reassociating halves of the chromosomes split in the preceding telophase. As the association becomes closer, the material of the threads is progressively concentrated, until it takes the form of a double spirem which segments into split chromosomes. These are separated into their component halves at anaphase and undergo a new splitting during telophase. Nuclei may go from the telophase of the last premeiotic division directly into the heterotypic prophase, or may pass through an intervening resting stage.

In the heterotypic prophase the reticulum gives rise to beaded "threads" which become more uniform spirems with a distinct parallelism, just as in the archesporial prophases. At this stage occurs synizesis, during which the reassociation of the parallel threads to form "filaments" is completed. From the contraction emerges a thick double spirem homologous with the double

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[1] DIGBY, L., On the archesporial and meiotic phases of *Osmunda*. Ann. Botany **33**:135–172. *pls. 8–12. fig. 1.* 1919.

spirem of the somatic prophase; the doubleness is believed to be the result of splitting in the last premeiotic telophase, and not to a conjugation of entire chromosomes. This double univalent spirem, which is more or less conspicuously beaded according to the fixing agent employed, is soon thrown into loops and the split becomes obscured. During the succeeding stages segments of the spirem (the "filaments"), although originally arranged end to end before segmentation, conjoin laterally in pairs to form the bivalent chromosomes, a process which is consummated in the second contraction. It is here that the conjugation of entire chromosomes occurs, whereas at the first contraction (synizesis) daughter halves of chromosomes are reassociated. As the second contraction loosens, the bivalents shorten and thicken and take up positions near the periphery of the nucleus (diakinesis). Only rarely at this stage can the temporarily obscured split of each component of the bivalent be detected.

As the bivalent takes its place upon the spindle, its univalent components become somewhat disjoined, and each again reveals the fission which had its origin in the last premeiotic telophase and was most conspicuous in the spirem of the early heterotypic prophases, and which marks the line of separation for the homotypic mitosis. As the univalent passes toward the pole, its halves widen out along this line of fission, giving the V-form characteristic of the heterotypic anaphase. During early telophase each daughter half of the split univalent undergoes a new longitudinal fission; this is homologous with the split occurring in the somatic telophase; after being obscured it reappears in the homotypic anaphase and functions in the post-homotypic division. The telophasic transformation of the chromosomes occurs as described for the archesporial divisions, and during interkinesis the individual chromosomes are indistinguishable.

The homotypic division is regarded as essentially a continuation of the last premeiotic division, since the doubleness of the chromosomes of the homotypic prophase is held to be the same as that of the last premeiotic telophase; the heterotypic division is consequently an interpolated process effecting numerical reduction. Although the events of the homotypic division are "involved in some obscurity," they seem to be in the main as follows. The threads derived from the fission of the daughter halves of the univalent chromosomes in the heterotypic telophase reassociate in pairs and form a number of chromatic masses, which later take the form of loosely associated daughter univalents; these arrange themselves more or less independently on the spindle. During their anaphasic separation (along the line marked out in the last premeiotic telophase) the fission which had its origin during the close of the heterotypic mitosis, and which is to function in the post-homotypic mitosis, reappears. The chromosomes at telophase take the form of double beaded threads which establish the resting reticulum as in the archesporial mitoses.

Although in substantial agreement with the conclusions of FARMER and MOORE,[2] this interpretation of maturation is directly opposed to that of GRÉGOIRE[3] and YAMANOUCHI,[4] who hold that the double heterotypic spirem in *Osmunda* arises from a conjugation of thin threads, each representing an entire chromosome, as stated in the first paragraph of this review. The GRÉGOIRE school charges the FARMER school with a misinterpretation of the presynaptic stages, while the latter charges the former with a neglect of the second contraction stages. It is not to be denied that the view stated fully by Miss DIGBY has certain advantages: it allows one interpretation to be placed upon the double spirem in both somatic and heterotypic prophases, irrespective of the exact time at which the split originates, and it also helps to explain the sudden appearance of the split for the second maturation mitosis in the anaphase of the first.

This question, however, must be settled primarily by direct evidence. It is obvious that its solution depends upon the exact manner in which the telophasic transformation of the chromosomes and the derivation of the latter from the reticulum in prophase are accomplished. It is granted by both sides that the alveolar or reticulate condition in which the chromosomes are found in late telophase is continuous with the similar condition seen in the succeeding prophase. If, therefore, it is true (1) that the telophasic transformation (alveolization) represents a true splitting, and (2) that the early prophasic reticulate condition passes directly into the double spirem, it follows that this doubleness in every prophase is due to the fission which originated in the preceding telophase, as held by Miss DIGBY. Contrary to the statement of that author, however, workers on mitosis are not at all generally agreed that the evolution of the chromosomes is that stated in (1) and (2). In his investigation of somatic mitosis in *Vicia Faba* for the purpose of elucidating these points, the reviewer,[5] contrary to the findings of FRASER and SNELL,[6] FRASER,[7] and others, showed not only that the telophasic alveolization is too irregular to permit of its being regarded as a splitting, but also that the reticulate condition of the prophase, instead of developing directly into the definitive split, gives rise to simple thin threads in which a new split develops. From

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[2] FARMER, J. B., and MOORE, J. E. S., On the meiotic phases in animals and plants. Quart. Jour. Micr. Sci. **48**:489–557. *pls. 34–41*. 1905.

[3] GRÉGOIRE, V., La formation des gemini hétérotypiques dans les végétaux. La Cellule **24**:369–420. *pls. 2*. 1907.

[4] YAMANOUCHI, S., Chromosomes in *Osmunda*. BOT. GAZ. **49**:1–12. *pl. 1*. 1910.

[5] SHARP, L. W., Somatic chromosomes in *Vicia*. La Cellule **29**:297–331. *pls. 2*. 1913.

[6] FRASER, H. C. I., and SNELL, J., The vegetative divisions in *Vicia Faba*. Ann. Botany **25**:845–855. *pls. 62, 63*. 1911.

[7] FRASER, H. C. I., The behavior of the chromatin in the meiotic divisions of *Vicia Faba*. Ann. Botany **28**:633–642. *pls. 43, 44*. 1914.

this it cannot be concluded that in no form does the split develop directly from the early reticulate structures, or that the telophasic alveolization, although irregular, may not later become so equalized as to constitute the first stages of the split; but it does follow that it is quite unsafe to use the principle of telophasic splitting as a premise from which to draw the conclusion that the approximation of thin threads in the early heterotypic prophase represents the reassociation of the halves of a single split chromosome. Although it is well to emphasize the importance of the premeiotic telophase, the ultimate solution of this perplexing problem must be reached mainly through a more refined analysis of those prophasic changes which have led a long list of investigators to the conclusion that the early heterotypic association of threads represents a conjugation of entire chromosomes which separate at the heterotypic division. To the reviewer the figures so far given by the English cytologists do not prove the theory they advocate.—L. W. SHARP.

Carbohydrate economy of cacti.—A distinct contribution to our knowledge of the carbohydrates in plants in general, and in the succulents in particular, is the report of SPOEHR'S investigations at the Desert Laboratory.[8] The methods employed give us what is probably the most complete analysis of the carbohydrates of a single plant tissue that we have, values for no less than 11 different groups of carbohydrates being ascertained, partly by direct determinations and partly by calculation.

The monograph is prefaced by a rather thorough discussion of carbohydrate metabolism in plants, and of the transformations of the carbohydrates under the influence of acid, alkali, oxidation, and enzymes, and of the energy relations of the products of these transformations. Then follows a description of the methods employed. *Opuntia phaeacantha* and *O. versicolor* furnished material for the studies. In preparing the tissues for carbohydrate analysis they were ground in a meat chopper and placed in an oven at 98° C. The precaution of DAVIS and DAISH of plunging the tissue into boiling alcohol was not deemed necessary. The disaccharides and polysaccharides were hydrolyzed by boiling with 1 per cent hydrochloric acid for 3 hours. All sugar determinations were made volumetrically with Fehling's solution. The pentoses were determined after fermenting away the hexoses with bakers' yeast.

The polysaccharides of the cactus are starch and xylan. The mucilage of *Opuntia* consists of 34.1 per cent d-glucose and 65.9 per cent l-xylose. Associated with it there is probably an acid. Glucuronic acid was found as a constituent of the sap. The formation of mucilage in special large cells could be watched under the microscope under certain conditions.

The relative abundance of the different groups of carbohydrates and also of water is profoundly affected by the seasonal variations of the external

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[8] SPOEHR, H. A., The carbohydrate economy of the cacti. Carnegie Institution of Washington. Publ. 287. pp. 79. 1919.

conditions. From the cool and humid winter to the hot and dry fore-summer the water content of normal species of *Opuntia* may change from about 80 to 65 per cent, and then rise again to 83 per cent during the humid but hot mid-summer. "Low water-content and high temperatures are associated with: (1) increase of polysaccharides; (2) decrease of monosaccharides; (3) increase of pentosans. High water-content and lower temperatures are associated with: (1) decrease of polysaccharides; (2) increase of monosaccharides; (3) decrease of pentosans." The author points out the significant fact that "the greatest activity of the plant comes at a time when the content of monosaccharides and disaccharides is highest," in March and April, although he is careful to state that a relatively large supply of simple sugars is not the only prerequisite for growth, but is only one of many factors.

In an arid atmosphere the cut joints undergo considerable decrease in water content, while still remaining normal in appearance and activity. The loss of water by transpiration and evaporation is partly compensated for by the water formed in the combustion of sugars, and partly by the condensation of the simple sugars into polysaccharides. Under drought the former decrease, while the latter and the pentosans increase, in total amount. The author suggests that the great imbibitional force of the pentosans may prevent the use of water for hydrolytic processes, when water becomes scarce in the tissue. These phenomena are closely correlated with temperature effects, when the latter are studied independently of varying moisture supply. Enzyme equilibria are discussed in connection with these two factors.

During the night the succulents respire sugar to acids, principally malic. This is not accompanied by an accumulation of alcohol. In an oxygen-free atmosphere, however, there is much less acid formed, and a very considerable amount of alcohol produced. One molecule of malic acid furnishes two of carbon dioxide and one of ethyl alcohol. Under these anaerobic conditions more sugar is consumed per unit of energy than under aerobic conditions. This is accompanied by an increase in the water content of the tissue.

During starvation the joints of *Opuntia* maintain the same relative proportions of the various carbohydrates. This disproves the theory that the pentoses are waste products of metabolism, since then they would show an increase. The water relations of the tissue during starvation and during periods of feeding on sugar solutions are discussed at some length.

SPOEHR advances the theory that the pentoses may be formed from glucuronic acid by the loss of a molecule of carbon dioxide, and discusses the isomerism relations between the hexoses and the corresponding pentoses that would be formed through the intermediary of glucuronic acid.—J. J. WILLAMAN.

Transpiration in tropical rain forests.—The lack of experimental data as to the conditions of plant growth and activity in tropical rain forests is apparently leading to some desirable investigation. A notable contribution in this

field is by McLean,[9] who worked in the rich forests on the slopes of the hills near Rio de Janeiro, Brazil. This is a region of high average humidity, due to a rainfall of 111.2 cm., the heaviest downfall being during the warmer months, and to a very considerable amount of cloudiness upon days with no rainfall. Considerable climatological data are presented, and a graph of climatic favorability is devised by combining the four factors of temperature, rainfall, relative humidity, and sunshine. The curve of this graph seems to show that the year may be divided into a more and a less favorable period, the latter extending from June to December.

Atmospheric humidity is shown to be high, even outside the forest cover. Graphs are presented showing the relative range of humidity and temperature at various levels of the vegetation. The latter records prove that a dense layer of shrubs divides the forest into two strata, the lower possessing cooler and more humid conditions than the lighter and better ventilated regions above. The author believes that this lower stratum is the less favorable to vegetation, and to it his experimental work is confined.

Transpiration measurements by means of potometers give the water loss by leaves in the lower stratum of the forest always less than 0.4 of the evaporation from a free water surface exposed alongside the foliage. Experiments within the laboratory with similar temperature and humidity, but with higher illumination, are shown to give similar results. Many of the shade leaves possess an amount of cutinization that reduces cuticular transpiration to a very slight amount. Structural studies show the intercellular spaces of sun and shade leaves to be relatively 16.3 and 24.8 per cent, and these amounts correspond very closely to those found in Europe. The size and amount of stomata seem to be rather decidedly smaller than that found in typical mesophytes of temperate lands. The vascular strands of the shade leaves are much smaller in cross-structure than those of sun leaves. These data, and the fact that the author believes the power of root absorption to be low, make it probable that, even in the protected region of the lower interior of the forest, transpiration may for short periods decidedly surpass the low capacity of the plants to supply water. This is supposed to account for cutinization, semi-succulence, and other xeromorphic tendencies and features of the tropical forests.

Under such conditions of reduced transpiration, however, there is no shortage of mineral matter, but on the contrary the leaves from shaded and protected habitats show relatively a richer content than do those sun forms with a much higher transpiration rate. This would prove that here at least the absorption of mineral salts is quite independent of any transpiration current.

A study of the foliage proves the predominance of the lanceolate leaf form and a remarkable prevalence of nyctitropic folding, which, however, does not

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[9] McLean, R. C., Studies in the ecology of tropical rain forests; with special reference to Brazil. I. Humidity. Jour. Ecol. 7:5–54. *pl. 1. figs. 21.* 1919.

seem to have a marked effect upon water loss. With the latter phenomenon is associated an abundance of pulvini.

The report is to be commended as an attempt to apply quantitative methods in an almost untouched field.—GEO. D. FULLER.

Heated soils.—JOHNSON[10] has done a very critical and exhaustive piece of work on the effect of heating soils at various temperatures on the germination of seeds and later growth of plants in such soils. The heating at 114–116° C. was done in an autoclave; at higher temperatures the heating was done with air-dry soils in dry ovens. The duration of heating was about 2 hours.

Soils heated at 100–115° C. gave temporary retardation of germination and seedling growth, followed later by a great increase in rate of growth. The extent of these varied greatly with the soil, seed, and plants used, and with other environmental conditions. The injury increased as the temperature rose up to 250° C. As the temperature rose above 250° C. the injury decreased until it was nil with heating at 350° C. or above. The time of recovery from the toxic effects was proportional to the intensity of the toxicity. Soils showed considerable variation in the degree of effect of heating. This variation cannot be explained on the basis of any one characteristic of the soil, but seems to result from a combination of a number of its characters.

Seeds varied in their sensitiveness. Lettuce and clover are very sensitive, and wheat, buckwheat, and flax are resistant. Gramineae and Cucurbitaceae are usually resistant, while Leguminosae and Solanaceae are more sensitive. There is great variation in the response of the growing plants. Heated soils that proved very injurious to some plants, as tomatoes, may be beneficial to others, as wheat. In general, but not always, there is a parallel between the sensitiveness of germination and of the later growth of the seedling. *Pyronema*, some other fungi, and some bacteria grow best in soils heated to 250° C., and fall off in growth rate with soils heated to higher or lower temperatures.

The ammonia content of soils is highest in those heated at 250° C., and diminishes as the temperature of heating rises or falls. The same is true of the concentration of the soil solution, so that there is a rough parallel between these characters of the soil and the degree of toxicity or later increased growth. Adsorptive capacity of the soil modifies the action of the toxic substance. In soil extracts the toxicity is more nearly correlated with the concentration of the ammonia. Additions of ammonia to soil produce effects similar to heating. The author believes the toxic action of heated soils is largely due to ammonia existing as ammonium carbonate. He thinks other factors are involved in so-called "chemical" injuries.

The toxic material in heated soils is volatile. It is also changed into non-toxic form when the soil is kept under conditions favoring growth of organisms. The latter is due to soil flora, and, contrary to PICKERING, does

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[10] JOHNSON J., The influence of heated soils on seed germination and plant growth. Soil Science 7:1–87. 1919.

not occur under aseptic conditions. The amount of ammonia apparently may increase as organisms reduce the toxicity. The ammonia is assumed in this case to exist in delicate transition stages detected by analysis, but not in toxic form. The soils heated above 250° C. are supposed to be less toxic because much of the ammonia is volatilized by the high temperatures.

The author believes that heating to very high temperatures does not change the quality of the effects gained by heating at ordinary sterilizing temperatures, but merely makes these effects more marked by quantitatively intensifying them. His results, therefore, are valuable in elucidating the effects of sterilizing soils by heat.—Wm. Crocker.

Vegetation of an antarctic island.—Lying 600 miles southwest of New Zealand, 920 miles southeast of Tasmania, and 970 miles from the antarctic continent, Macquarie Island is in a position of great isolation. It is little more than a short range of mountains with peaks ranging from 900 to 1424 ft. in height, the length of the island being 21 miles and its breadth less than 4 miles. The hills descend rapidly toward the sea, forming bold headlands and precipitous cliffs with no harbors or sheltered bays. It possesses a remarkably equable temperature, the mean maximum being 43°.5 F. and the mean minimum 37°.9 F., while the extreme range is only 25°.8 F. A rainfall of 45 inches is distributed so that no month has less than 3 inches. Wind velocity is uniformly great, averaging 18 miles per hour.

It has an impoverished vascular flora of 30 seed plants, 3 ferns, and 1 lycopod. Concerning the origin and affinities of this flora, Cheeseman[11] decides that with the exception of 3 endemic grasses it dates back no farther than the last glacial epoch. Its repopulation was probably affected through the agency of birds, as half its species are common to New Zealand, 15 are found also in Fuegia or South Georgia, and a like number are circumpolar.

The vegetation is characterized by the entire absence of trees and shrubs. The conspicuous plant forms are the tussock grasses, principally *Poa foliosa*, the large leaved "Macquarie Island cabbage," *Stilbocarpa polaris*, an Araliaceous plant resembling a fine rhubarb, the cushion of *Azorella Selago*, globular mosses often 4 ft. across, and a purple flowered Composite, *Pleurophyllum Hookeri*, with long sage green leaves. Of these the tussock grass is most abundant, occupying much of the hillside slopes.—Geo. D. Fuller.

Journal of the Arnold Arboretum.—This new quarterly journal has been established to secure "the prompt publication of information about trees and shrubs collected at the Arnold Arboretum," which was a function of *Garden and Forest* (1887–1897). The first number (July 1919) includes the fifth paper of Camillo Schneider entitled "Notes on American willows" (pp. 1–32);

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[11] Cheeseman, T. F., The vascular flora of Macquarie Island. Sci. Rep. Australian Antarctic Expedition of 1911–14. Series C. vol. 7. pt. 3. pp. 63. map. 1919.

"New species, varieties, and combinations from the herbarium and the collections of the Arnold Arboretum," by ALFRED REHDER (pp. 44–60); "A phytogeographical sketch of the ligneous flora of Korea," by E. H. WILSON (pp. 32–43); and the fifth paper by C. S. SARGENT entitled "Notes on North American trees" (pp. 61–65).—J. M. C.

Toxicity of alpha-crotonic acid.—Alpha-crotonic acid, in concentrations of 25–50 p.p.m., is very toxic to wheat plants. Its toxicity is markedly reduced by the phosphate radical, as SKINNER and REID[12] show by using it in water cultures of wheat with a three-salt medium varying according to the triangle system. The crotonic acid does not affect the relative absorption of any one salt, thus differing from some of the other toxic organic compounds studied in SCHREINER'S laboratory. The real nature of the antagonism is not known.—J. J. WILLAMAN.

New genera.—NAKAI[13] has described a new genus of Oleaceae (*Abeliophyllum*), found in Corea. It is an endemic and related to *Fontanesia* (Fraxineae), a monotypic oriental genus.

PENNELL[14] has described a new genus of Onagraceae (*Peniophyllum*), based on *Oenothera linifolia* as the type. In a conspectus of *Kneiffia* (*Oenothera*) he recognizes 13 species, 4 of which are described as new.—J. M. C.

Plant mucilage.[15]—The mucilage in cacti, mallows, tragacanth, and lilies arises in special large cells by hydrolysis of the cellulose wall, a hydrocellulose being an intermediate stage. These walls are not secondarily thickened. An account is given of the reaction of these mucilages to various stains.—J. J. WILLAMAN.

Germination.—RUSSELL[16] finds that the germination of camphor seeds in the commercial seed bed is greatly improved by removing the pulp. By pulping the seeds the increase in the number of seeds of transplantable size amounted to 60 per cent.—WM. CROCKER.

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[12] SKINNER, J. J., and REID, F. R., The influence of phosphates on the action of alpha-crotonic acid on plants. Amer. Jour. Bot. **6**:167–180. 1919.

[13] NAKAI, TAKENOSHIN, Genus novum Oleacearum in Corea media inventum. Bot. Mag. Tokyo **33**:153, 154. 1919.

[14] PENNELL, F. W., A brief conspectus of the species of *Kneiffia*, with the characterization of a new allied genus. Bull. Torr. Bot. Club **46**:363–373. 1919.

[15] LLOYD, F. C., Origin and nature of the mucilage in the cacti and in certain other plants. Amer. Jour. Bot. **6**:156–166. 1919.

[16] RUSSELL, G. A., Effect of removing the pulp from camphor seed on germination and the subsequent growth of the seedling. Jour. Agric. Research **17**:223–238. 1919.

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

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 259

H. L. WALSTER

(WITH EIGHTEEN FIGURES)

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. The 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 thermo-regulators. 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

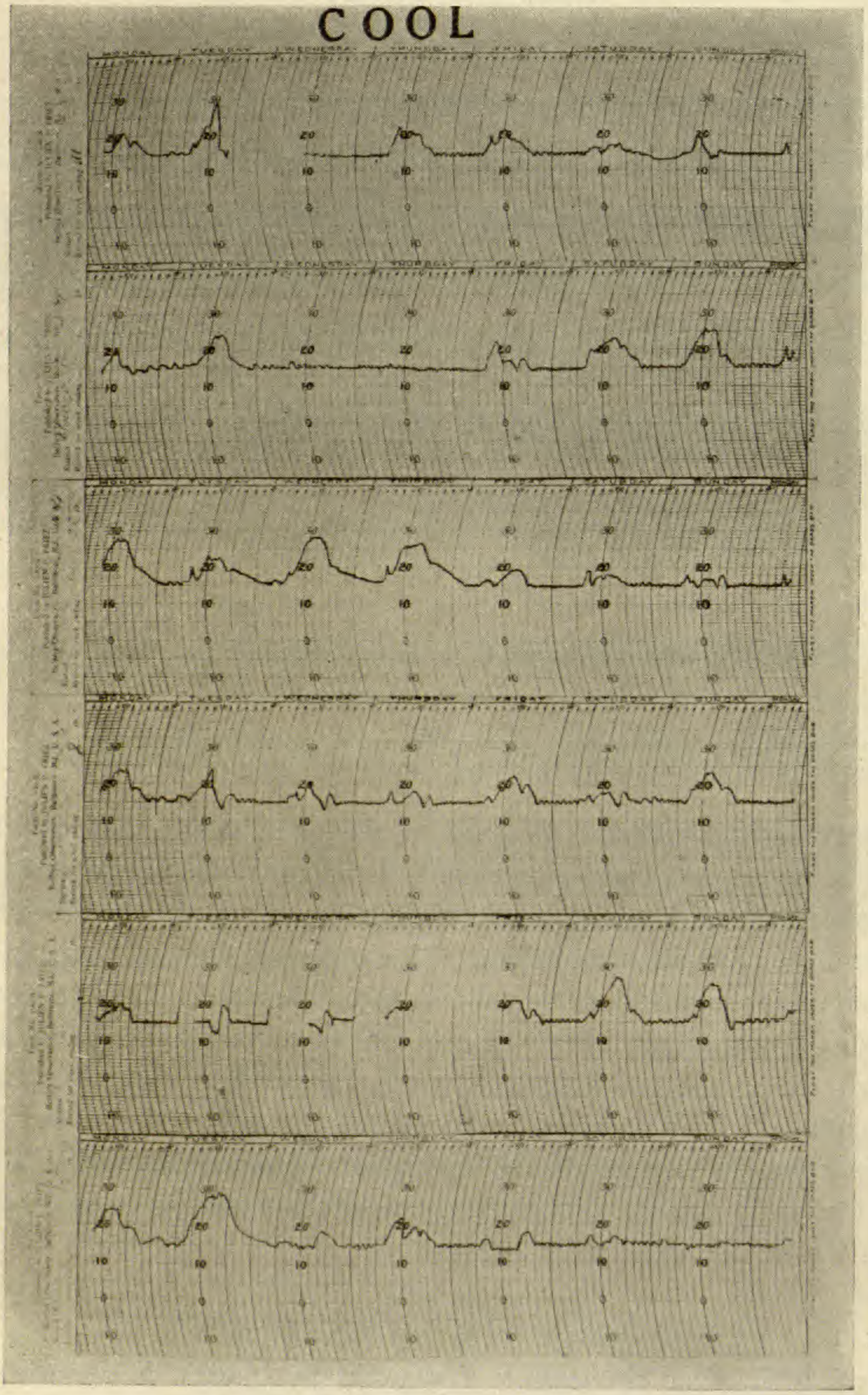

FIG. 1.—Thermograph records showing air temperature in cool house from planting to time of sampling for chemical analysis

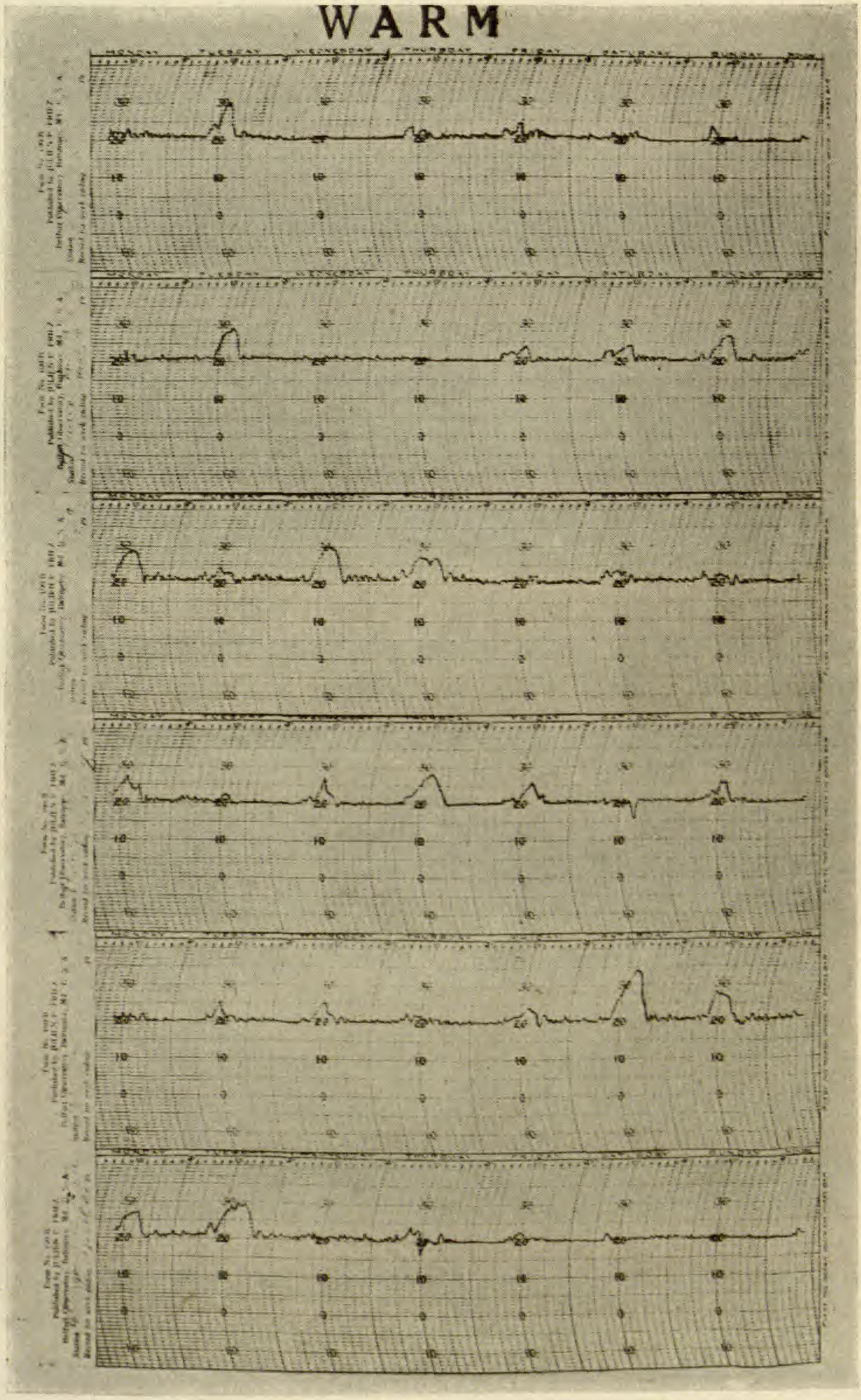

FIG. 2.—Thermograph records showing air temperature in warm house from planting to time of sampling for chemical analysis

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 total 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.

AGRICULTURAL COLLEGE
N.D.

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PHYSIOLOGICAL STUDY OF MAPLE SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 260

H. A. Jones

(WITH TWO FIGURES)

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,

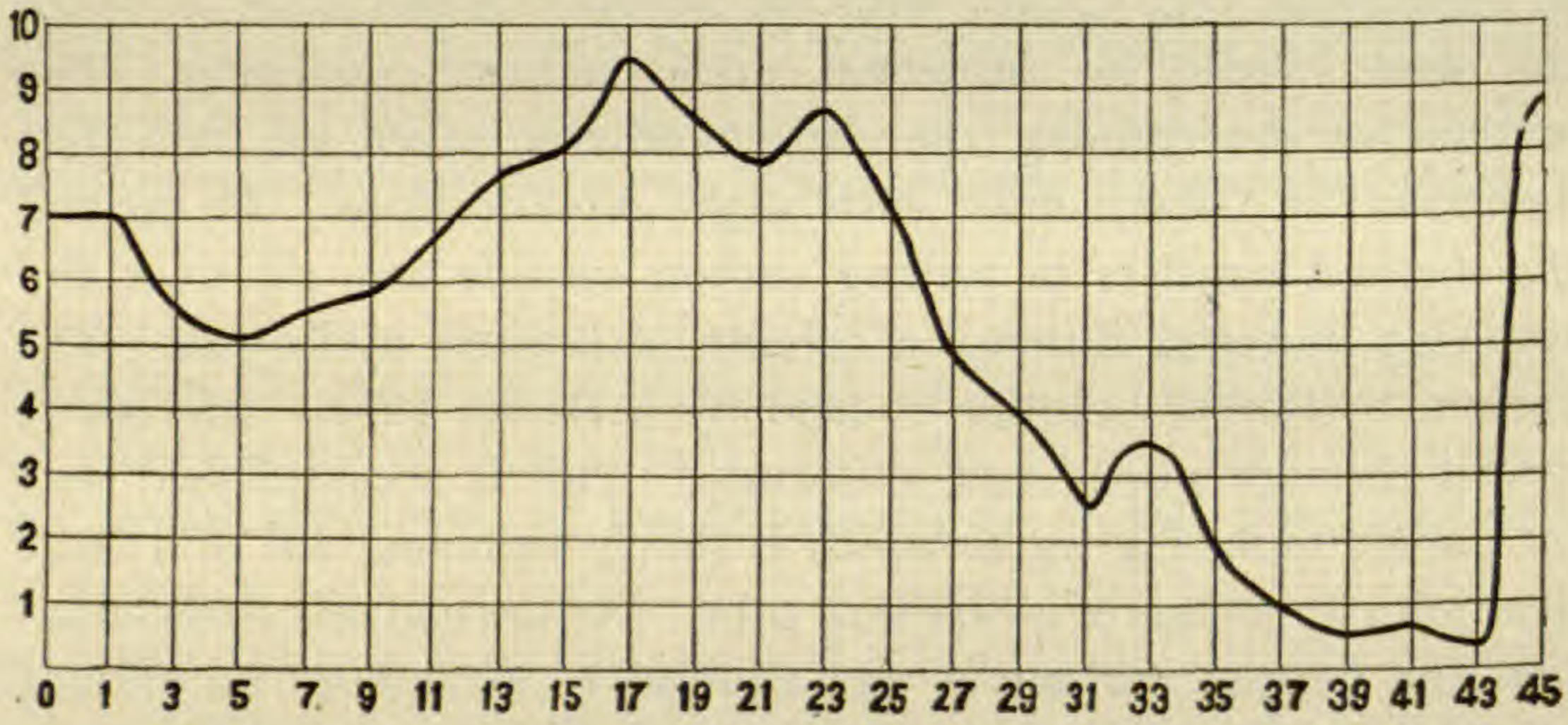

FIG. 1.—Respiration curve for seeds desiccating at 25° C.; mg. of CO_2 given off in 24 hours per gm. dry weight plotted on ordinates; time of desiccation in days plotted on abscissae; great rise in respiration after forty-third day due to placing desiccated seeds (dead at time) under favorable germinative 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

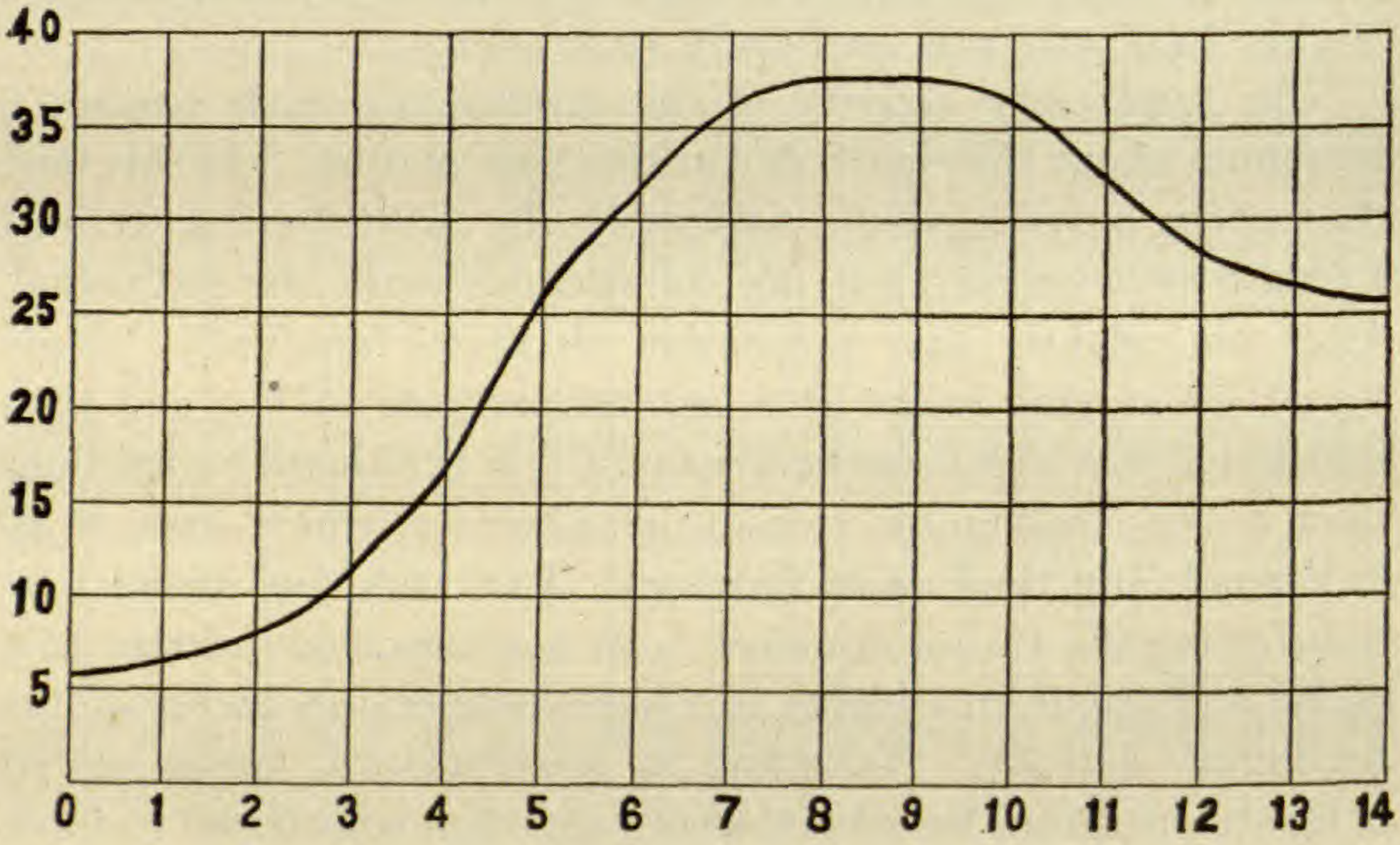

FIG. 2.—Respiratory curve for first 14 days of germination in dark at 25° C.; time of germination in days plotted on abscissae and mg. of CO_2 given off in 24 hours per gm. of dry weight plotted on ordinates.

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 0° 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 Roseceae. 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

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JOHN T. BUCHHOLZ

(WITH FIFTEEN FIGURES)

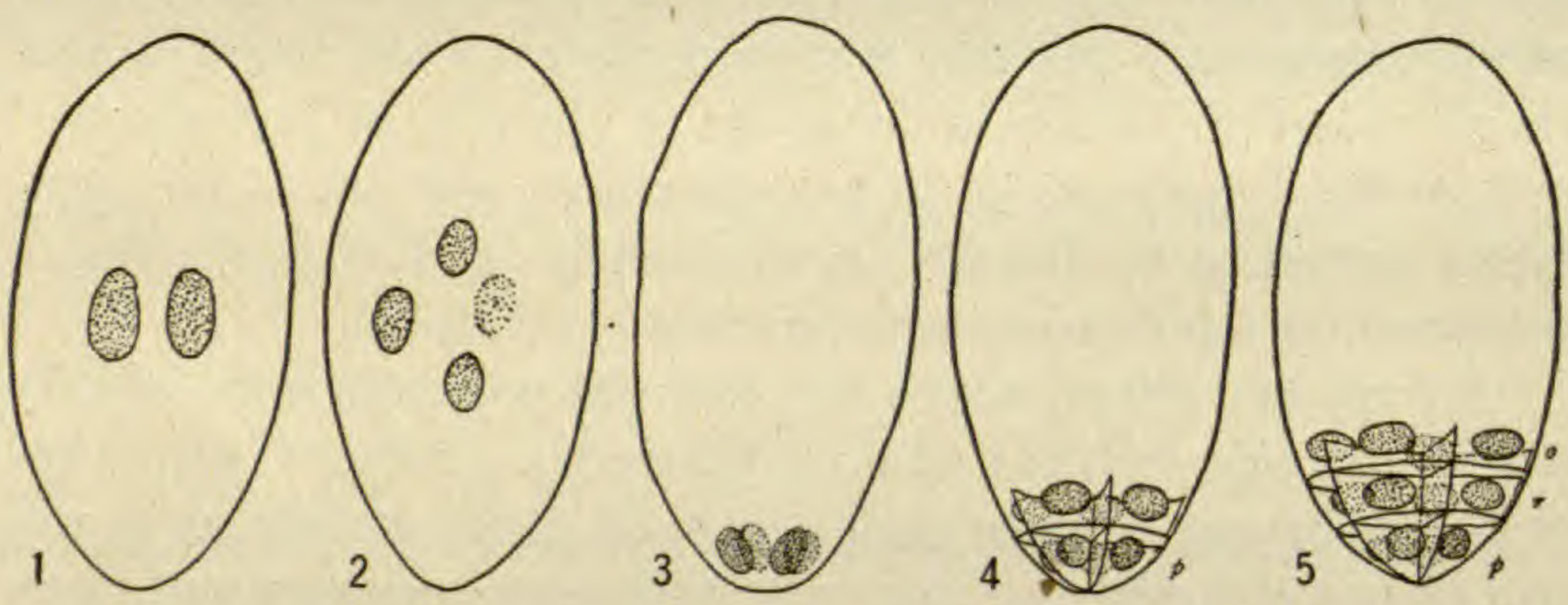
Polyembryony among conifers is of two kinds: cleavage polyembryony, in which a single fertilized egg gives rise to many embryos; and the simple polyembryony, which is due to plurality of archegonia. This latter form is encountered wherever there are several eggs that may be fertilized, and therefore 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.



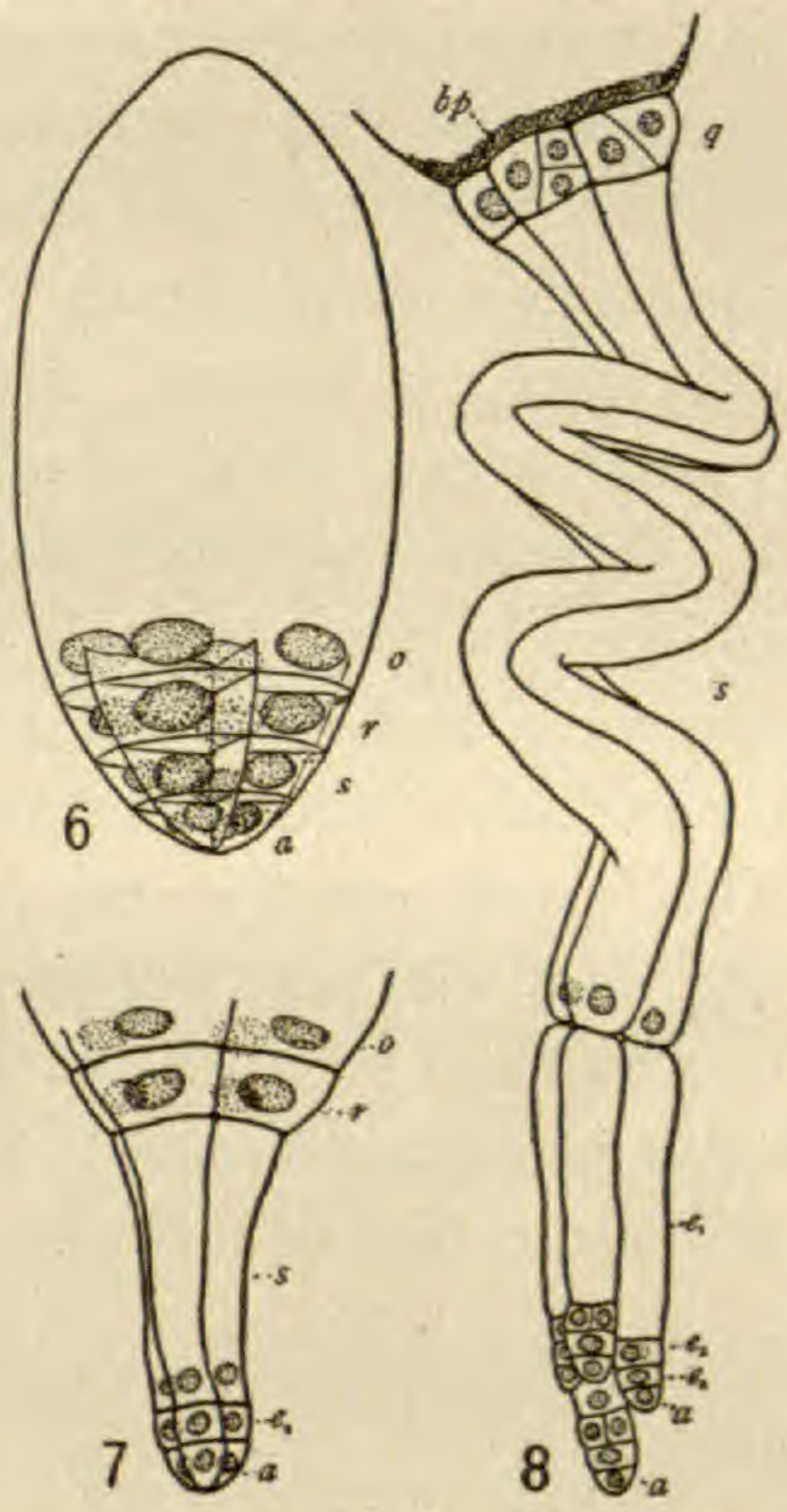
FIGS. 1–5.—Steps in development of proembryo in *Pinus*, diagrammatic reconstructions from serial sections and published figures: *p*, tier of primary embryo initial cells; *r*, tier of rosette cells, initial cells of rosette embryos; *o*, upper open tier of cells; normally tiers *r* and *o* come from 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.

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.



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.

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.

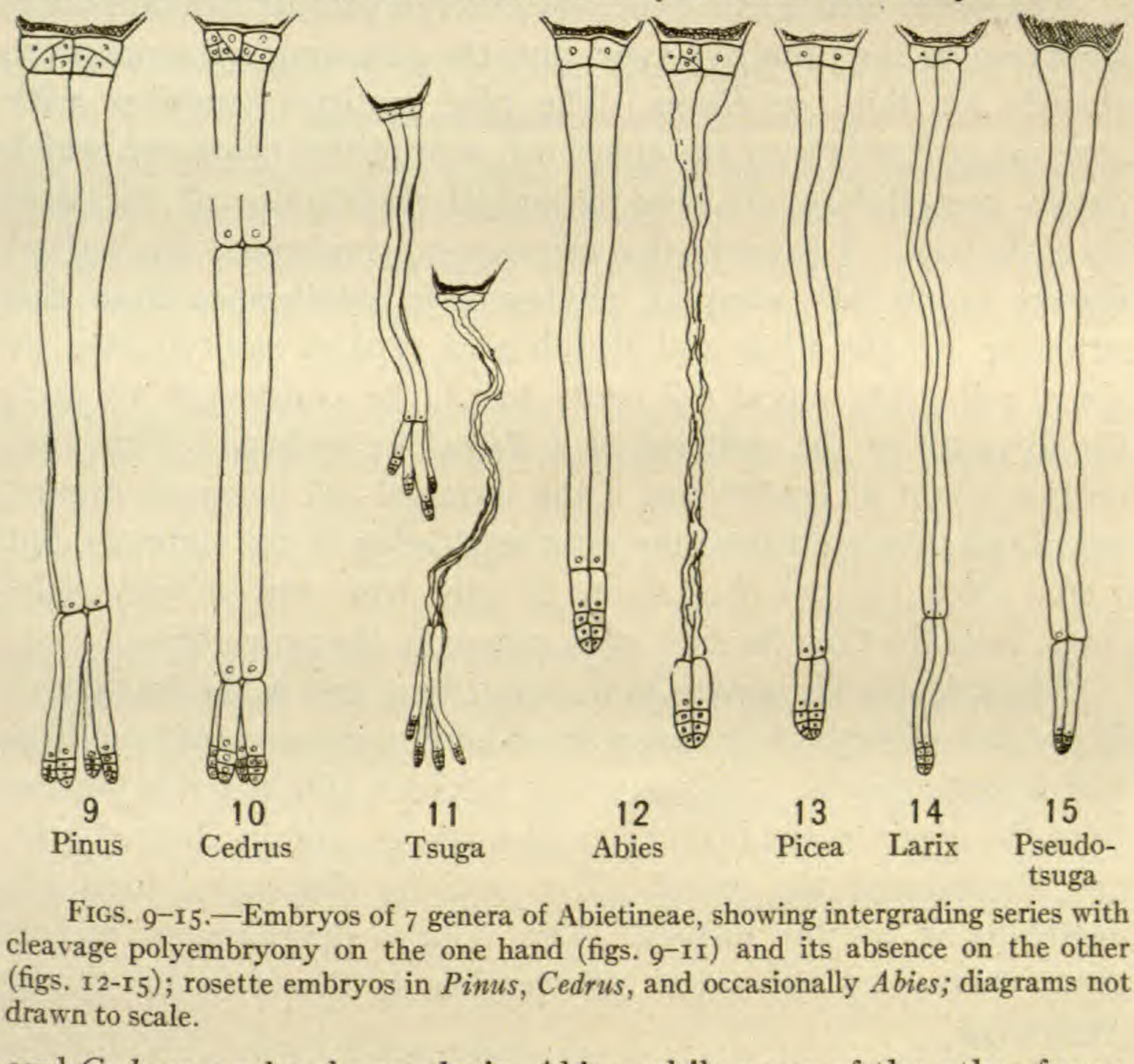
Although the available material of *Larix* was also somewhat limited, several outstanding features may be described with certainty. Like *Picea* and *Abies*, only one embryo is produced per archegonium. Except for the different appearance in size and proportion, the embryo of *Larix* is very similar 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 Abïetineae.

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

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 262

THOMAS G. PHILLIPS

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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POSITION OF SCUTELLUM AND HOMOLOGY OF COLEOPTILE IN MAIZE

(WITH ELEVEN FIGURES)

PAUL WEATHERWAX

The homologies of the grass embryo and their bearing upon ideas of the phylogenetic relationship of monocotyledonous and dicotyledonous plants have been subjects of study and discussion for a long time, and although most botanists are fairly well agreed upon most phases of the question, some points are still subject to controversy. It is realized that evidences drawn from a single species as highly specialized as maize will not go far toward the making or the breaking of a theory, but two things have been observed in the structure and development of the embryo of *Zea Mays* that seem to have a definite bearing upon the subject, and these are offered for what they may be worth.

The history of the subject has been fully reviewed, and certain sharply contrasted opinions have been presented recently by WORSDELL and by COULTER and LAND. Further reference to the voluminous literature seems unnecessary here, and only those points to which the information at hand is related will be considered.

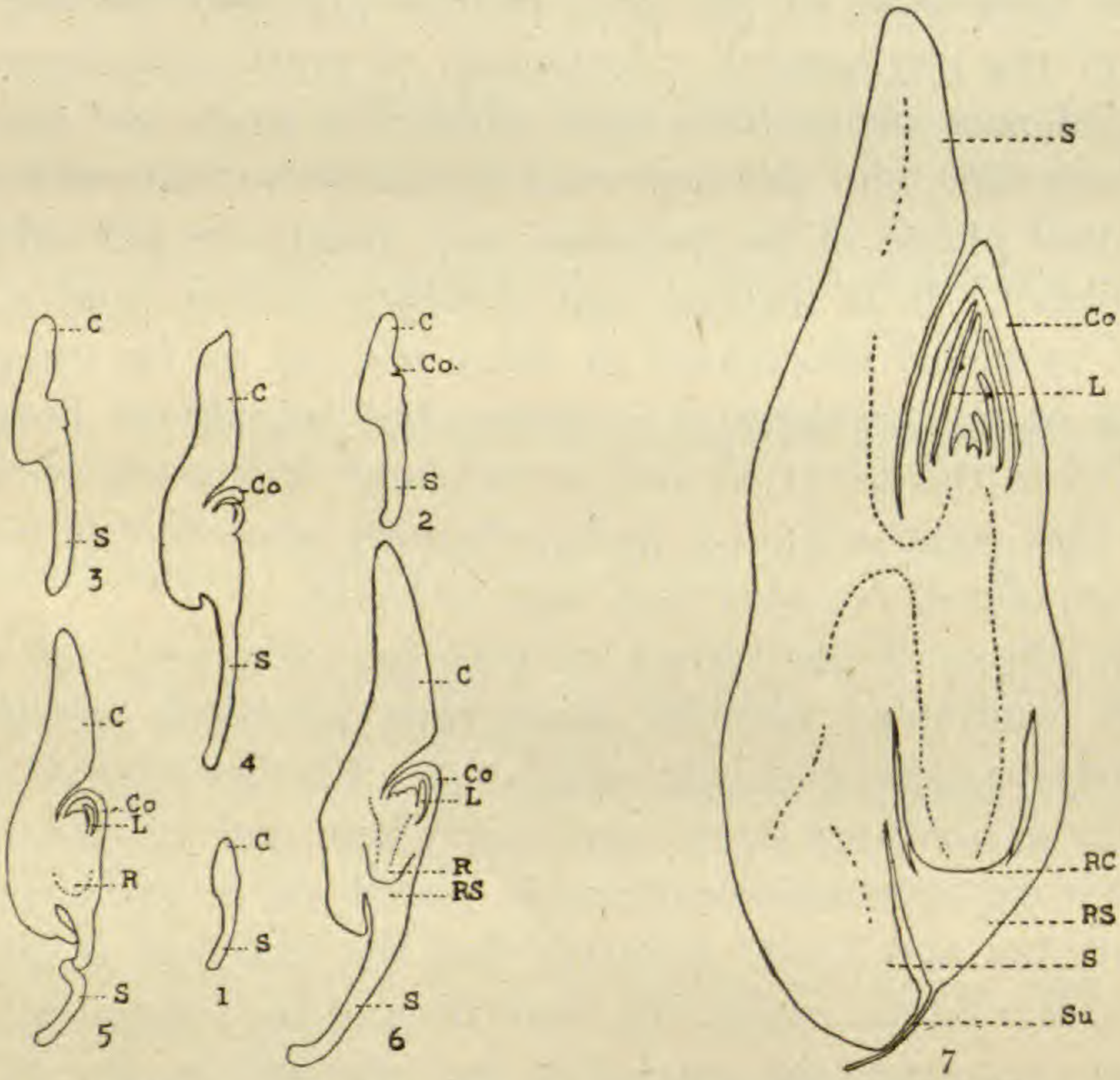
COULTER and LAND[1] maintain that the scutellum of the grass embryo is a lateral organ, the equivalent of the foliage leaf. The epiblast represents the cotyledon that was lost in the evolution from the monocotyledonous condition, and the coleoptile is the third leaf. Opposed to this is WORSDELL'S[2] contention that the cotyledon, which he considers terminal in origin, is the lamina, and the coleoptile is the ligule, of a single foliage leaf, whose sheath was present only in early stages of development. The epiblast is said to be the equivalent of the auricles of the foliage leaf. The principal evidences brought to the support of this view are the double

[1] COULTER, J. M., and LAND, W. J. G., The origin of monocotyledony. II. Monocotyledony in grasses. Ann. Mo. Bot. Gard. **2**:175–183. 1915.

[2] WORSDELL, W. C., The morphology of the monocotyledonous embryo and that of the grass in particular. Ann. Botany **30**:509–524. 1916.

nature of the vascular system of the coleoptile, the bifid character of the epiblast in some grasses, and the forked coleoptile found in a few seedlings of maize. As a background for these details is the idea that the monocotyledonous condition is the primitive one.

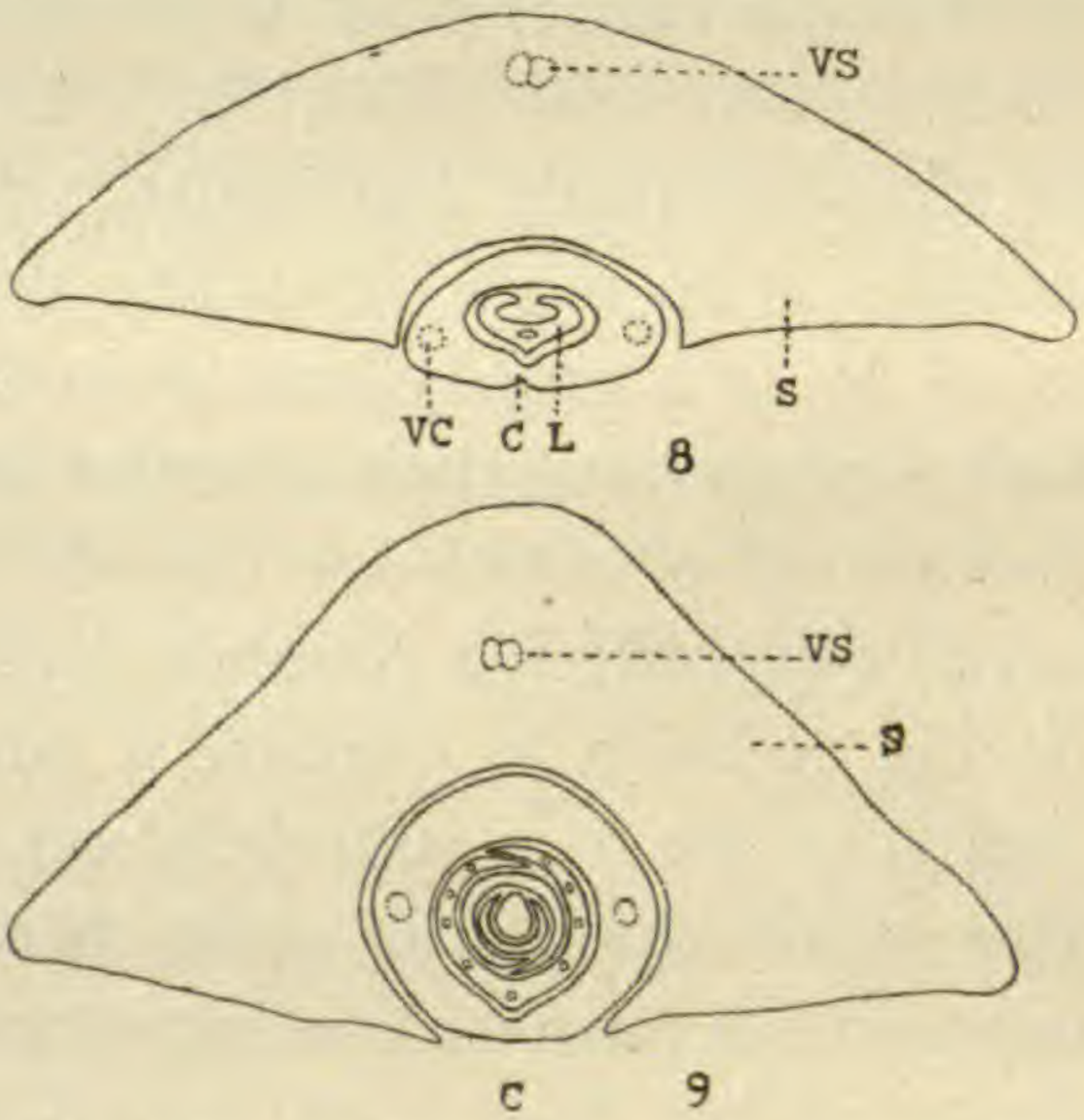
The first piece of evidence that I have to offer on these questions is in the form of a series of steps in the development of the embryo of maize (figs. 1–7). These stages have often been observed and

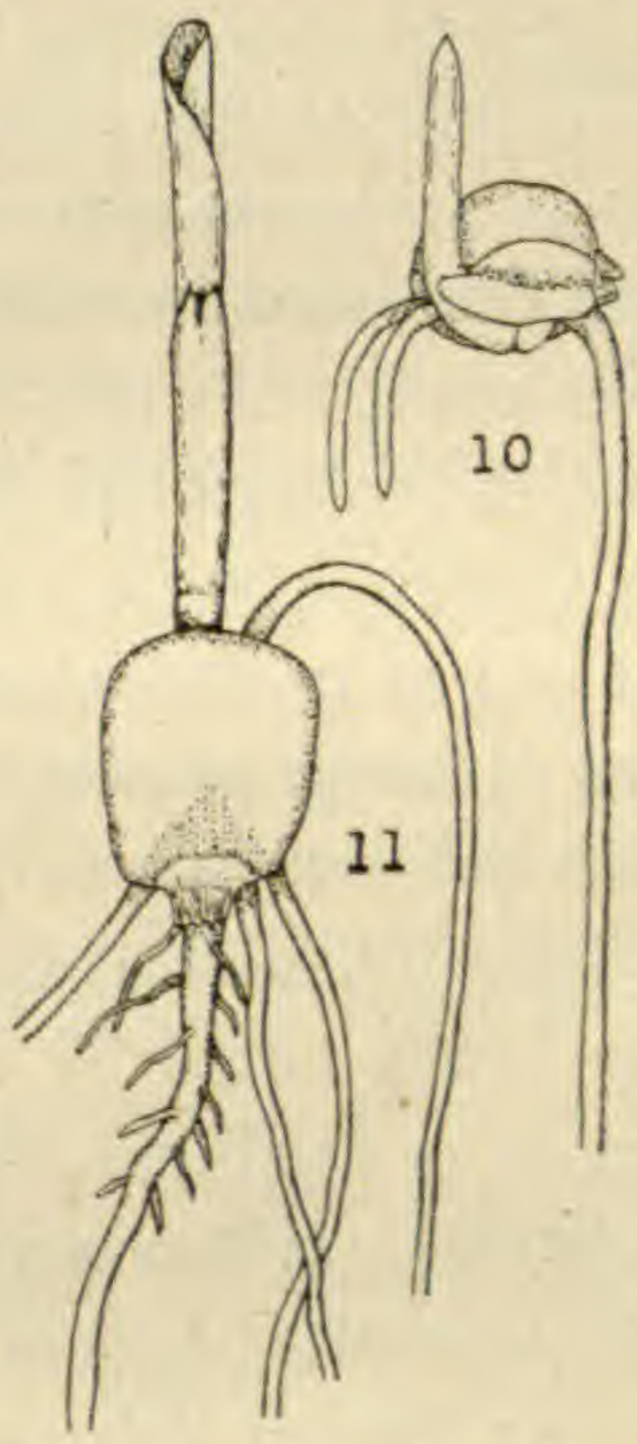


FIGS. 1–7.—Figs. 1–6, steps in development of embryo: *C*, cotyledon; *S*, suspensor; *Co*, coleoptile; *L*, foliage leaf; *R*, root; *RS*, root sheath; ×15; fig. 7, longitudinal section of nearly mature embryo: *S*, scutellum; *Co*, coleoptile; *L*, foliage leaf; *RC*, root cap; *RS*, root sheath; *Su*, suspensor; ×15.

discussed more or less abstractly, but I have failed to find a complete series figured. In so far as appearances may be trusted, no evidence is clearer than this series. The appearance of the mature embryo (fig. 7) leaves little doubt of the terminal position of the plumule, and preceding stages of development bear this out fully; the cotyledon is never terminal, even in the earliest stages. As soon as the young embryo begins to differentiate, so that anything

that may be called a cotyledon is visible (fig. 1), the whole structure has an asymmetrical form due to the more rapid development laterally of the cotyledon, and subsequent steps emphasize this (figs. 2–7). That the coleoptile is at first directed horizontally or downward, as is emphasized by WORSDELL, is of little significance; morphological position cannot always be determined geometrically. Moreover, WORSDELL'S[3] figures, taken from another authority in





FIGS. 8–11.—Figs. 8, 9, transverse sections of embryo through plumule: *VS*, vascular strands of scutellum; *VC*, vascular strands of coleoptile; *L*, foliage leaf; *C*, point of union between two sides of coleoptile, forming closed sheath; sections of embryo of liguleless variety in no essential way different from these; ×15; fig. 10, germinating seed of liguleless maize: coleoptile present and normal; fig. 11, seedling of maize, showing forked coleoptile.

substantiation of his position, are only the upper parts of embryos; if we attach to the figures the lower parts of the corresponding stages of development of the embryo of any typical grass, the continuity of cotyledon, hypocotyl, and suspensor as the axis of the embryo is evident.

The second point in support of the view taken by COULTER and LAND is afforded by the embryo of a liguleless variety of maize isolated by EMERSON a few years ago. These plants are like those of ordinary maize, except that they breed true for the absence of

[3] *Ibid.*, fig. 3, A-E, p. 511.

ligules and auricles. A few of the plants tend to produce at least rudimentary ligules on the uppermost leaves, but they are regularly absent from the lower leaves, and the condition might reasonably be expected to extend to the cotyledon also. An examination of the embryo and of the seedling, however, shows the coleoptile to be present and normally developed (figs. 8–10). While this fact cannot be accepted as a proof of anything, it should at least not be overlooked in considering the question.

WORSDELL has probably given undue emphasis to the arrangement of the vascular strands of the coleoptile and to the forked tip of this organ in some seedlings of maize (fig. 11). It is true that the coleoptile has two vascular strands bilaterally placed (figs. 8, 9), while the foliage leaf has several strands equally distributed; but this modification in vascular anatomy is no more significant than that shown in the scutellum (figs. 8, 9), in a prophyllum, or in the palea of many grasses, all of which tend to have their vascular elements present in two groups, and yet all of which are considered modified foliage leaves. The forked coleoptile is a common occurrence often noted by anyone having occasion to examine a large number of seedlings of maize, and it is due to a superficial set of conditions. The coleoptile begins to develop as an open sheath (figs. 2–4), the edges of which soon unite to form a closed structure; but the line of this union is always visible (figs. 8, 9), especially near the top of the sheath. Too much significance must not be attached to the nature of the mechanical rupture of this structure by the elongating plumule. If the union of the two sides has not been very firm, and it usually is not, the structure will split on one side only; but if the two sides are firmly grown together, the coleoptile may split for a short distance down two sides, producing the forked coleoptile (fig. 11). The relation between this occurrence and the duplex ligules of some grasses, or the two stipules of some other plants, is too remote to merit consideration.

It may be said, therefore, that the evidences derived from the structure and development of the maize embryo, including that of the liguleless mutant, favor the idea that the coleoptile is the homologue of a foliage leaf, and that the cotyledon is a lateral organ.

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CURRENT LITERATURE

NOTES FOR STUDENTS

Ecological terms and concepts.—Ecology, one of the latest branches of botanical science, has naturally an immature and incomplete terminology, although several of its followers have made attempts to remedy the latter defect. Some of the recent discussions of problems of terminology are worthy of note, not so much for their contributions to nomenclature, as to their logical division of the subject and their criticism of the mistakes of the past. This seems particularly true of an article by PAVILLARD,[1] in which he begins with a historical and critical sketch of conditions in the past, and proceeds with an analysis of the scope of plant geography. Here it is suggested that it would be desirable for all to follow the practice of the Swiss school and employ the designation "geobotany" suggested by GRISEBACH in 1866. Two main divisions of the science are then made, resting upon the two fundamental units of the species and the association. It is further suggested that the latter be appropriately termed plant sociology or phytosociology, and that the problems of each division be segregated into three categories, giving as the subdivisions of the subject: (1) Floristic geobotany, (2) Genetic geobotany, (3) Ecologic geobotany, (4) Floristic phytosociology, (5) Genetic phytosociology, and (6) Ecologic phytosociology. Whether this classification be universally adopted or not, it has much to recommend it in logical clearness, and, further, it shows considerable agreement with the best usage of the past.

The content of floristic geobotany would remain the same as for floristic plant geography as delimited by WARMING in 1895, while ecologic geobotany would not differ materially from the autecology of SCHRÖTER in giving emphasis to the relationship of the individual species to its habitat and the growth form by which it responds to its environment. To genetic geobotany would be referred such questions as the geographical aspect of the origin of species, heterogenesis, and endemism.

In the division devoted to problems connected with the plant association, the use of the term phytosociology or plant sociology was proposed by JACCARD in 1910, and employed in a more limited sense by HARPER.[2] The second and third subdivisions seem about equivalent to SCHRÖTER's synecology, genetic

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[1] PAVILLARD, J., Les progrès de la nomenclature dans la géographie botanique. Ann. Geogr. **27**:401–415. 1918.

[2] HARPER, R. M., The new science of plant sociology. Sci. Monthly **4**:456–460. 1917.

phytosociology being almost exactly the same as COWLES's[3] physiographic ecology, while ecologic phytosociology corresponds very closely to WARMING'S ecological plant geography. To floristic phytosociology would be referred not only the enumeration of the flora of the associations, but also more exact studies as to the importance of the species to the community, and the constancy of the relationship. These relationships are discussed in detail by PAVILLARD[4] in a more recent paper. One phase of this relationship has been estimated in a quantitative manner by BRAUN-BLANQUET,[5] by giving to each species in an association a coefficient of affiliation (Gesellschaftstreue), the first rank (5) being conferred upon species confined exclusively to the particular association, and the lowest (0) belonging to ubiquists. To this PAVILLARD adds another evaluation of the species, based upon its importance in development and maintenance of the association, and expressed as its genetic coefficient, and here also the numerical value is also from 5 to 0. Analyzed in such a manner, the floristic composition seems to PAVILLARD decidedly the best manner of characterizing an association. The characterization of the plant association by floristic composition only is also insisted upon by DU RIETZ and his associates.[6] They also favor attention to priority in the use of ecological terminology, a concession that ecological writers are not likely to grant. DU RIETZ contends that the Swedish school of ecologists is distinguished by the use of true inductive methods as contrasted with the less desirable procedure of other workers. He also proposes certain new terms of minor importance.

GAMS[7] is less modest in his demands, for he wishes to abolish the use of formation, association, and most other synecological (or biocoenological) terms now current, because they have been and still are being employed in different senses by different writers. Instead of such fairly familiar terms, he would substitute a new set founded to some extent on new concepts. He contends that two types of units, the ecological and topographical, have been confused and should be distinguished with care. The former he calls "synusia" (associations), and distinguishes three grades where the component elements

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[3] COWLES, H. C., The physiographic ecology of Chicago and vicinity. BOT. GAZ. **31**:73–86. 1901.

[4] PAVILLARD, J., Remarques sur la nomenclature phytogéographique. Montpellier. pp. 27. 1919.

[5] BRAUN-BLANQUET, J., Eine pflanzengeographische Excursion durch Unterengadin und in dem schweizerischen National Park. Bericht. Schw. Bot. Gesells. **26**:1–79. 1918.

[6] DU RIETZ, C. E., FRIES, T. C. E., and TENGWALL, T. A., Vorschlag zur Nomenklatur der soziologischen Pflanzengeographie. Svensk. Bot. Tidskrift **12**:145–170. 1918.

[7] GAMS, H., Prinziprenfragen des Vegetationsforschung. Ein Betrag zur Begriffsklarung und Methodik der Biocoenologie. Vierteljahrschr. Naturf. Gesells. in Zurich **63**:293–493. 1918.

of the unit are respectively (1) of the same species, (2) of different species but of the same growth forms and of similar aspect, and (3) of different species and various growth forms presenting different series of aspects but united into an ecological unit in a single habitat by fixed correlation. This last grade of synusium corresponds very nearly with the "association" of most authors. Similar synusia are grouped as "isocies." For the topographical unit he adopts the word "biocoenose" (or biocoenosium), and uses it for the vegetation of a unit habitat. Biocoenosia of different regions which are compounded of isocies are called "isocoenosia."

The author rejects all attempts to classify vegetation units upon dynamic lines. He also gives a new classification of life forms, based largely upon the RAUNKIAER system, but more extended and including animals. It is safe to predict that such revolutionary changes as those urged by GAMS, even if they are logically conceived, will not be acceptable to the ecologists of America, and, judging from the criticism of the scheme by PAVILLARD (1919), they will meet with no greater favor in France.—GEO. D. FULLER.

Statistical methods in ecology.—It seems appropriate that from among the students of that father of modern ecology, EUGENE WARMING, should come a leader of perhaps the most promising line of advance in the ecology of today. RAUNKIAER more than any other has opened the way for the introduction of quantitative methods in the study of vegetation. His method of comparing the floras of different regions by means of a numerically expressed biological spectrum,[8] and of evaluating the mesophytism of a habitat by leaf classes,[9] have been noted in this journal. The latter method of estimating vegetation was made more familiar to American ecologists by the translation of FULLER and BAKKE,[10] who also included in their article a summary of a statistical method that had been familiar to Danish readers for some years.[11]

In a more recent article RAUNKIAER[12] has summarized the material of his former contributions, and has been able to show something of their applications to the solution of ecological problems. His statistical or valence method consists in determining the relative abundance of the different species composing a plant community of definitely limited extent, called by him a "formation," although more nearly equivalent to an association as understood by American ecologists. This determination is made by taking a census of a

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[8] BOT. GAZ. **51**:309–310. 1911.

[9] BOT. GAZ. **63**:242. 1917.

[10] FULLER, GEO. D., and BAKKE, A. L., Raunkiaer's life-forms, leaf-size classes, and statistical methods. Plant World **21**:25–37, 57–63. *fig. 1*. 1918.

[11] RAUNKIAER, C., Formations undersgelse og Formationsstatistik. Bot. Tidskr. **30**:20–132. 1909.

———, Om Valensmetoden. Bot. Tidskr. **34**:304–311. 1917.

[12] ———, Recherches statistiques sur les formations végétales. Det. Kgl. Danske Videnskabernes Selskab. Biol. Meddeleser. I **3**: pp. 80. *figs. 3*. 1918.

number (25–50) of small unit areas of the vegetation, selected at random or according to fixed plans, and outlined by the revolution of a metal radius of determined length attached to a walking stick.[13] The convenient size of these unit areas appears to be 0.1 sq. m., and the frequency with which a given species appears in such areas determines its valence, frequency percentage, or frequency coefficient. Emphasis is placed upon the fact that in an undisturbed area the vegetation will eventually come to a practically complete equilibrium with the factors of the habitat, and will be composed of the species of the region best fitted to exist under such conditions. RAUNKIAER therefore defines his "formation" as "essentially homogeneous from a floristic point of view," that is, homogeneous as to the dominant species or the species showing the highest frequency coefficients. Such a statistical method permits the quantitative comparison of similar plant communities and their more exact delimination.

It is interesting to note as the results of the use of such statistical methods, principally the examination of many plant communities involving the determination of over 8000 coefficients, that 55 per cent of the species have coefficients ranging from 1 to 20, 15 per cent from 81 to 100, 14 per cent from 21 to 40, 9 per cent from 61 to 80, and 3 per cent from 41 to 60. In other words, the least frequent species in the communities studied were most numerous, while the most frequent came second in order of number of species, with a much smaller number showing moderate abundance. These phenomena the author expresses in the form of a law. "In a formation in a relative state of equilibrium what allows one or more species to prosper at the expense of their neighbors is the fact that the dominant species are better adapted to live under the conditions existing within the formation of which they are a part and by their community life ('concurrence vitale') they prevent the other species from equaling them in frequency. But however well equipped they may be for such community life, they are not able to prevent other species, widely disseminated but fewer in individuals, from entering the formation and occupying portions that for any reason whatever may have been left unoccupied by the dominant species. Thus we see that there is a much larger number of the least frequent species."

For the further analysis of vegetation RAUNKIAER describes a method of arriving at the area occupied by each species in the community. This is accomplished by the study of unit areas similar to those employed in the determination of frequency; indeed the two could be done simultaneously. To assist in readily determining the portion of the area occupied by the areal parts of a species he adds a series of radii of determined length to the one already affixed at right angles to the walking stick. These are so spaced that they divide the circular unit area into fifths and tenths, so that by their aid

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[13] RAUNKIAER, C., Measuring apparatus for investigations of plant formations. Bot. Tidskr. **33**:45–48. 1912.

the observer is easily able to estimate 10 different degrees of covering. From a record of the numbers representing these degrees of covering the areal percentages of the different species are readily established.

A summary of the methods employed, and a classification of vegetation upon the basis of life-forms and leaf-sizes, completes an article rich in suggestions to the ecologist seeking more accurate methods.—GEO. D. FULLER.

Susceptibility gradients.—Following his demonstration of axial metabolic gradients in animals and their relation to the course of development and individuation, CHILD entered upon a study of axiate plants, particularly the algae. His first paper[14] on axial gradients in algae appeared several years ago. His interesting and valuable observations[15] have been extended to include a considerable number of new forms, and the results are sufficiently uniform to warrant the general conclusion that plants and animals are essentially similar in respect to these axial susceptibility gradients.

Twenty-five species have been studied, 14 of which were considered in the earlier paper, and all of them show an axial gradient in susceptibility to injury and death from such agents as KCN, alcohol, ether, HCl, $HgCl_2$, $CuSO_4$, neutral red, temperature, etc. When killing concentrations are used, death occurs first in the apical region and proceeds basipetally in each axis. The susceptibility gradient is a general indicator of metabolic rates, death occurring soonest in the most active protoplasm. The susceptibility gradient is rather easily altered or reversed by external conditions, by advancing age, physiological isolation of cells and branches, and other factors. The ease with which such reversals occur indicates in some degree the sensitiveness of species.

He finds[16] that the unicellular and multicellular hairs, either branched or unbranched, which occur on some algae, possess the same kind of axial gradients as the main axis. In such forms as *Fucus* and *Castagnea*, in which the hairs have basal growth, the gradient is acropetal; but whenever the hairs grow apically the normal gradient is basipetal. Reversals may be induced in these hairs, also, especially with low concentrations of the susceptibility reagents. In some cases the agent may reverse the susceptibility to itself, or one agent may reverse the susceptibility to another agent. These results indicate clearly that hairs represent physiological axes, and the gradient of susceptibility appears to be one of the aspects of physiological polarity in all axes. When the axial gradients are reversed, these hairs often separate into their component cells, or the hairs drop from the main axes. Loss of hairs in laboratory material

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[14] CHILD, C. M., Axial susceptibility gradients in algae. BOT. GAZ. 62:89–114. 1916.

[15] ———, Further observations on axial susceptibility gradients in algae. Biol. Bull. 31:419–440. 1916.

[16] ———, Susceptibility gradients in the hairs of certain marine algae. Biol. Bull. 32:75–92. 1917.

is undoubtedly associated with reversed gradients brought about by unfavorable conditions of confinement.

These changes in gradients of hairs were studied particularly in *Griffithsia.*[17] If conditions are not extreme, obliteration or reversal of the axial gradient is followed by cell separation, and the death of some of the cells, the death-rate being higher among isolated apical cells than among those more basally situated. The cells which do not die usually proceed to grow new apical cells, which are found to arise at the most susceptible end of the old cells. This is usually the *basal* end, because the normal gradient had been reversed before the cells were disconnected. Rhizoids, however, arise only on those parts of the cell which have the lowest metabolic rates or lowest susceptibility.

The general conclusion of all this work is summarized admirably in the words of the author: "The facts support the conclusion that a gradient in metabolic rate, protoplasmic condition, or whatever we prefer to call it, of which the susceptibility gradient is within certain limits an indicator, constitutes physiological polarity in protoplasm, and that such a gradient is not an inherent property of protoplasm, but may be determined and altered by external factors."

Students who desire to repeat some of these experiments for themselves will find a recent paper of interest.[18] The axial gradient may be very beautifully demonstrated colorimetrically by the use of dilute solutions of potassium permanganate. The protoplasm reduces the permanganate and takes on a brown color, which appears first and deepest in the most active regions. Concentrations of M/1000 to M/100,000 should be used for such experiments.—C. A. SHULL.

Biology and culture of the higher fungi.—Among recent contributions to our knowledge of this difficult subject is a paper by BOYER[19]. The first part deals with attempts at spore germination and culture of over 60 species, and the second gives in more detail the results of his work with *Morchella* and *Psaliota*.

He recognizes three types of higher fungi: (1) pure saprophytes, (2) facultative parasites, and (3) mycorhizal forms which are constantly associated with certain trees. Saprophytes, he finds, grow readily on culture media, and many give rise to carpophores; while many of the mycorhizal group cannot be grown in pure cultures on any of the many types of media tried. Between pure saprophytes and forms which will not grow on culture media he finds

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[17] CHILD, C. M., Experimental alteration of the axial gradient in the alga *Griffithsia Bornetiana*. Biol. Bull. **32**:213–233. 1917.

[18] ———, Demonstration of the axial gradients by means of potassium permanganate. Biol. Bull. **36**:133–147. 1919.

[19] BOYER, G., Études sur la biologie et la culture des champignons superieurs. pp. 116. *pls. 4. figs. 20.* Bordeaux. 1918.

gradations in dependence upon the mycorhizal habit. Some will make only a very slight mycelial growth in cultures, while others will form abundant mycelia, but never develop carpophores. Field experiments also confirm this mycorhizal dependence, but attempts to trace mycelium from carpophore to tree were seldom successful. He considers the mycorhizal relationship to be symbiotic, the green plant furnishing carbohydrates and in return receiving water and salts, especially nitrogenous substances which the fungi probably obtain by the fixation of free nitrogen.

As a source of cultures he first tried the germination of spores. Various media and methods of treating spores were tried, but no germinations from mycorhizal forms such as tubers or amanitas were obtained, and from other forms the mycelium obtained was seldom vigorous. Because of this he resorted to the use of portions of the carpophore, flamed over a Bunsen burner, as a source of cultures, and found this (which he erroneously considers a new process) much more satisfactory. In this manner he obtained cultures of 24 species which he describes, giving figures for 17 of them. While many media were used, he found a decoction from carrots, solidified with gelose (a gum derived from agar-agar), the most satisfactory. Cultural variations bring into question the validity of some specific characters, such as size, color, and characters due to substratum.

In his studies of *Morchella* cultures were obtained from single spores. The mycelium was very vigorous, growing well at 10–12° C. Sclerotia 0.5–4 mm. in diameter appear in 10–15 days. No conidia or ascocarps were formed. He attributes the absence of ascocarps either to the limited mycelial growth in cultures, or, as he considers more probable, to the necessity of a mycorhizal host prior to ascocarp formation.

Cultures obtained from the spores of *Psaliota* were always weak, while those from portions of the carpophore were very vigorous. From his pure cultures he easily developed successful commercial spawn. Cultures from one carpophore always developed carpophores with the same varietal characters as the original, which is a great practical advantage.—LEVA B. WALKER.

Identification of mahoganies.—To meet the need of some adequate method for distinguishing the different commercial timbers now classed as mahoganies, DIXON[20] has prepared (1) a concise working definition of the term mahogany, and (2) an anatomical key accompanied by detailed descriptions for the identification of some of the more common kinds by means of their microscopic characters. The constant increase in the number of species of mahogany-yielding trees in economic use, and the doubtful authenticity of many of the specimens derived from commercial sources, have made the construction of such a scheme of classification most difficult.

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[20] DIXON, H. H., Mahogany, the recognition of some of the different kinds by their microscopic characteristics. Notes from the Bot. School, Trinity College, Dublin 3:58. *pls. 22–54.* 1919.

The first part of this preliminary paper discusses the many varied properties of these different woods, with regard to color, density, hardness, presence or absence of year-rings, pore-rings, size and contents of vessels, distribution of parenchyma, etc., and also the numerous contradictory definitions of mahogany to which these structural differences have given rise. To the general public and to the majority of woodworkers, mahogany is a reddish wood, generally with some distinct figure and texture, and valued in proportion to the beauty of its figure and the resistance of the wood to splitting and warping. Obviously such a definition is not sufficient. Reddish color and figure, both emphasized as distinct diagnostics of the original mahogany, *Swietenia mahogoni*, of course are essential, as also is the character described as "roeyness." According to DIXON, we may recognize as mahogany "all red or red-brown timbers in which the fibers of the adjacent layers cross each other obliquely, and so give rise to a play of light and shade on longitudinal surfaces ('roe'), greatly emphasizing the figure and conferring on the wood a freedom from splitting and warping." In addition, a mahogany should have scattered vessels, isolated or in small radial groups; the circumvasal parenchyma should be thin, and the medullary rays not more than 9 cells in width and under 2 mm. in height. In other respects the different woods designated by this name exhibit great structural variability.

The second part of the article presents the key and well written anatomical diagnoses of Western, African, Asiatic, and Australasian mahoganies. The 23 plates are from photomicrographs of transverse, radial, and tangential sections of the various woods, and are intended to show their distinct microscopic features.—LADEMA M. LANGDON.

Comparative salt absorption.—STILES and KIDD[21] have published two papers on the mechanism of salt absorption by disks of carrots and of potato tubers. Their method of study was to immerse a quantity of uniform disks of the material in salt solutions, and follow the course of absorption by the changes in the electrical conductivity. Although the conductivity is affected, not only by absorption of salt, but also by exosmosis, the writers believe that the latter is small, especially in the case of carrot. Potassium, sodium, and calcium chlorides are readily absorbed in all concentrations from N/10 to N/5000. The initial rate of absorption is roughly proportional to the concentration, but after a time this does not hold. The ratio of final internal concentration (arrived at by calculation) to final external concentration they call the absorption ratio. With low external concentrations this ratio is many

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[21] STILES, W., and KIDD, F., The influence of external concentration on the position of the equilibrium attained in the intake of salts by plant cells. Proc. Roy. Soc. B **90**:448–470. 1919.

———, The comparative rate of absorption of various salts by plant tissue. Proc. Roy. Soc. B **90**:487–504. 1919.

times unity, but with higher concentrations it becomes considerably less than unity. Although this relation can be expressed by the adsorption formula $y=kc^m$ (y is the final internal, c the final external concentration, and k and m are constants), the writers do not feel the data justify the conclusion that absorption of these salts is an adsorption phenomenon.

Kations are absorbed initially in the order K, [Ca, Na], Li, [Mg, Zn], Al; as equilibrium is approached the order is K, Na, Li, [Ca, Mg]. The initial order for the anions is SO_4, NO_3, Cl; the final order, NO_3, Cl, SO_4. "Although TROENDLE'S view, that in any group of the periodic classification the metallic ions are absorbed more rapidly the higher the atomic weight, is not contradicted, yet the view that the initial rate of absorption is largely dependent upon the mobility of the ions or diffusibility of the salt is equally well supported, and can be put forward provisionally as a more reasonable hypothesis."

Another paper, by STILES and JÖRGENSEN,[22] is polemical with THODAY, concerning the method of estimating the osmotic pressure of sap by the swelling or shrinkage of the tissue when immersed in salt solutions. Using sections of the root of the red beet, they found that they neither gained nor lost in weight in 0.40 N NaCl, and that this concentration was also just insufficient to cause plasmolysis. The writers therefore maintain that this concentration is *approximately* isotonic with the beet root sap.—J. J. WILLAMAN.

Tyrosin in fungi.—DODGE[23] reports some investigations on the chemistry of the tyrosinase reaction in the fungi which turn blue or black on exposure to air. The fungi were sliced, dried, and then ground into a flour, and this fungus flour used in the investigation. "In the work with tyrosin, the dried fungus flour was added directly to the substrate, toluol added, and the mixture left to extract the enzym and the enzym to react with the tyrosin." The author studied the reactions with the amino, carboxyl, and phenol groups. A modified form of the "micro" VAN SLYKE apparatus was used for the determination of the amino nitrogen, the permutite method of FOLIN and BELL for the determination of ammonia, and the colorimeter method of DUGGAR and DODGE for the determination of the carboxyl and phenol groups.

The following conclusions are drawn from these investigations: "(1) that the tyrosinase reaction is not a deamination, although it is possible that deaminases may exist in the same organism with tyrosinase; (2) that the tyrosin molecule is synthesized into a larger, more complex molecule, in which part of the carboxyl groups is either split off as carbon dioxide, or more probably bound in the molecule so that it will not react with alkali."—J. WOODARD.

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[22] STILES, W., and JÖRGENSEN, W., On the relation of plasmolysis to the shrinkage of plant tissue in salt solutions. New Phytol. **18**:40–50. 1919.

[23] DODGE, C. W., Tyrosin in the fungi: chemistry and methods of studying the tyrosinase reaction. Ann. Mo. Bot. Gard. **6**:71–92. 1919.

Cytology of gigantism.—The relation between the nuclei, and particularly the chromosomes, of exceptionally large individuals or varieties of a species has been described in several cases. TISCHLER[24] secured a giant form of *Phragmites communis* var. *Pseudodonax* which reached a height more than double that of the usual form. A comparison of the reduction divisions in the pollen mother cells of *P. communis* and the var. *Pseudodonax* brought him to the conclusion that, in this case, the gigantism arises through an increase in the size of the chromosomes, without any increase in their number. Other cases have been described in which the gigantism is due to an increase in the number of chromosomes, as in some forms of *Oenothera*, *Primula*, and *Solanum*.

The relation between chromosomes and dwarfing has received little attention from botanists, but the cytology of *Oenothera Lamarckiana* var. *nanella*, as described by GATES, and some observations by zoologists, indicate that the dwarfing is correlated, sometimes with a decrease in the number of chromosomes, and sometimes with a diminution in their size, without any change in their number.—C. J. CHAMBERLAIN.

Ecology of fossil plants.—In a report upon some fossil plant material found in the gorge of the Columbia River, in Oregon and Washington, CHANEY[25] notes that some 80 species are represented, 75 of which are angiosperms, of which 2 only are monocotyledons. A list of the genera with the number of species in each includes: *Ginkgo* 1, *Pinus* 1, *Smilax* 1, *Cyperacites* 2, *Populus* 3, *Salix* 3, *Hicoria* 2, *Juglans* 1, *Alnus* 1, *Carpinus* 1, *Corylus* 1, *Castanea* 1, *Quercus* 12, *Ulmus* 2, *Planera* 2, *Magnolia* 1, *Laurus* 2, *Platanus* 2, *Liquidambar* 3, *Crataegus* 1, *Sterculia* 1, *Rhus* 1, *Ilex* 1, *Acer* 3, and *Fraxinus* 1. From a study of this material the author concludes that the climate indicated by this Eagle Creek flora appears to have been somewhat warmer and drier than at present. The length of the epoch is to be placed at thousands rather than at scores of years. The dominant plants point to the existence of two habitats, one xerophytic and the other mesophytic. An area of upland dissected by a valley furnishes such habitats, and at the same time meets the geological requirements of the formation.—GEO. D. FULLER.

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[24] TISCHLER, G., Untersuchungen über den Riesenwuchs von *Phragmites communis* var. *Pseudodonax*. Ber. Deutsch. Bot. Gesells. **36**:549–558. *pl. 17*. 1918.

[25] CHANEY, R. W., The ecological significance of the Eagle Creek flora of the Columbia River gorge. Jour. Geol. **26**:577–592. *figs. 3*. 1918.

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SHORT CYCLE UROMYCES OF NORTH AMERICA[1]

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(WITH PLATE X)

The short cycle *Uromyces* may be segregated as a group by utilizing the criteria of life cycle and character of teliospores. Aside from any question of the validity of such bases for segregation, it is evident that it is a common practice thus to set apart this group, and that an opportunity is thereby afforded to consider relationships of such rusts to each other and to other rusts.

The short cycle *Uromyces* are of considerable interest, although as yet comparatively few species or even collections are recorded for North America. These rusts occur over a wide geographical range, however, and are parasitic upon widely separated families of hosts.

The writer has been privileged to examine the excellent collection of short cycle *Uromyces* in the herbarium of Dr. J. C. ARTHUR. This paper represents the results of the study of the group as made primarily for the *North American Flora*, but is presented separately in order to give notes and discussions not permissible in that publication.

[1] Abstract submitted before American Phytopathological Society at the New York meeting, and published in Phytopathology **7**:74. 1917. Contribution from Botanical Department of Purdue University Agricultural Experiment Station.

Characters and relationships

The rusts considered in this paper are those which fulfil the following requirements:

Cycle of development includes only pycnia (sometimes) and telia, both subepidermal.

Pycnia deep-seated, globose or flask-shaped, with ostiolar filaments.

Telia erumpent, usually grouped; teliospores free, pediceled, 1-celled; wall firm, colored, smooth or variously sculptured; germination by a single promycelium from an apical pore.

Urediniospores normally absent but occasionally found in the telia.

The association of pycnia with telia has for some time been considered the criterion of short cycle rust (1, 4). The occurrence of definite aecia or uredinia (providing the evidence indicates that the aecia or uredinia belong with the telial stage present) suffices to exclude a specimen from the group under consideration. While some suspicion may be aroused by the presence of urediniospores, such spores occasionally occur in the telia. In the cases in which pycnia are only rarely or not at all produced, telia only being present, the arrangement and character of the telia usually may be utilized to indicate whether or not the specimen is short cycled; a grouping of the telia in definite circinating or crowded groups, or the occurrence of germination of the teliospores at or soon after maturity, usually means that such a specimen belongs with the group of rusts here treated. In certain cases, however, the telia are diffused, and other considerations must be brought to bear.

A study of the *Uromyces* forms of the rusts as represented in the Arthur herbarium and of the literature indicates that, in North America, only the 11 species described are at present known to belong in reality to short cycle forms.

DIETEL (10) pointed out that the percentage of endemic species of rusts is higher in proportion to the isolation of the geographical region; that *Uromyces* shows a higher percentage of species in warmer than in colder regions; and that in both the Old and the New World the number of species of *Uromyces* is about one-third that of the number of species of *Puccinia*. It is to be noted that, so far as known, 8 of the 11 short cycle species of *Uromyces* are endemic to North America, and only 1 of the 11 species occurs also in Europe.

These forms are more especially found in the warmer parts of the continent, just as all *Uromyces* seem to be more numerous in warmer regions. While in North America some three times as many species of *Puccinia* as of *Uromyces* exist, the relation of the forms when divided according to their life cycle is strikingly different; for about 140 short cycle species of *Puccinia* are known for North America, in contrast to these 11 species of *Uromyces*.

P. and H. SYDOW (**25**), in their monograph of *Uromyces*, described only the telial stage for 183 of the 504 species considered in that work. For only a very few of these, however, were pycnia described. When full information is in hand, a large number of the 183 forms will of course be found not to be short cycled. It appears, however, that a comparatively greater preponderance of these short cycle forms of *Uromyces* may be found in the more tropical regions. The observation of MAGNUS (**17**) and of FISCHER (**15**), that increased altitude results in shortened life cycles for the rusts, is somewhat borne out by the fact that certain short cycle *Uromyces* are limited to the Rocky Mountain region. The effect of altitude and temperature can be better noted with the more numerous short cycle species of *Puccinia*.

ORTON (**21**) has touched upon the relation of a group of rusts with a common life cycle, opsis forms of *Puccinia* (the genus *Allodus*), to other groups with different life cycles. Comparable relationships and correlations with other rusts are to be noted with the group of rusts considered in this paper; some attention is directed to these points with the discussion of the several species. The rust in its development is intimately dependent upon its host. FISCHER (**13**) in 1898 emphasized the similarity between the teliospores of certain short cycle rusts and of long cycle heteroecious rusts whose aecia occurred upon the host of the short cycle form. He considered that this similarity indicated a phylogenetic relationship between such rusts with different life cycles. DIETEL (**9**) considered that the Uredinales have probably developed during geological times along with their hosts. ARTHUR (**5**) has pointed out that the relationships of the rusts often reflect the relationships of the hosts upon which they occur. The writer (**6**) has also dealt somewhat with this point.

DIETEL (11) considered that *Uromyces* is the most primitive of the Pucciniaceae, both on account of the possession of 1-celled teliospores, and because it occurs upon such diverse families of monocotyledons and dicotyledons. Whether long cycle or short cycle rusts are more primitive is still a mooted question.

The existence of species of these rusts as lepto-forms or micro-forms, that is, whether or not the teliospores germinate upon maturity, while subject somewhat to seasonal variation, is a fairly constant and characteristic feature with each species.

Life history; cytology

The life cycle is simplified in a short cycle species to the extent that only telia, often with pycnia, are produced. The occasional occurrence of a few urediniospores in the telia is a phenomenon in common with other groups of rusts which ordinarily do not bear such spores.

FISCHER (12) first cultured a short cycle *Uromyces*. He sowed teliospores of *U. Cacaliae* (DC.) Unger upon *Adenostyles alpina* Kern, securing telia again without the intervention of any other spore stages. No trace of pycnia was found. In 1905 FISCHER (14) reported the culture of the short cycle species of *Uromyces* which occurs in Europe as well as in America, *U. Solidaginis*. He sowed teliospores upon *Solidago Virgaurea alpestris*, and in about 13 days noted the infection upon the leaves; telia were produced, but in no case were pycnia to be observed. While North American material of this species has not been cultured, it is supposed that similar conditions obtain here. SCHNEIDER (24) cultured *U. Scillarum* (Grev.) Wint., a short cycle form, and reported specialization as to hosts. The teliospores were found to be capable of germination, either at once or after a period of rest. No cultures of endemic North American short cycle *Uromyces* seem to have been reported. CARLETON (8), ARTHUR (3), and others, however, have reported cultures of some species of lepto-*Puccinia*. WILLE (27) recently found a sharp specialization of the lepto-form *Puccinia Arenariae* upon the different host genera attacked.

The evidence obtained from cultures indicates that similar conditions exist in the short cycle forms, both of *Uromyces* and *Puccinia*.

A greater specialization and fixity may exist with short cycle forms than with forms with long life cycles; of course fewer spore forms upon which variability may be manifested are present.

Pycnia may be produced, under certain conditions, in some of these short cycle species of *Uromyces* not yet known to produce pycnia. It is to be noted, however, that species in which the teliospores germinate at maturity, that is, lepto-forms, seldom produce pycnia. Teliospores cannot function directly as repeating spores, but in lepto-forms a comparatively rapid repetition is secured through the intervention of the basidiospores, which are produced immediately upon maturity of the teliospores.

Cytological work upon the short cycle rusts indicates that similar conditions obtain with the short cycle species, both of *Uromyces* and *Puccinia*. The work of SAPPIN-TROUFFY (**23**) upon the histology of the rusts included a study of the short cycle forms *Uromyces Ficariae* (Schum.) Lev. and *Puccinia malvacearum* Mont. His observations were corroborated and extended with the two rusts, among others, by BLACKMAN and FRASER (**7**). They found that the general vegetative mycelium of *Uromyces Ficariae* consists of uninucleate cells, some of the later vegetative, together with the sori-forming, mycelium being binucleate. They found similar conditions for *Puccinia malvacearum*, the binucleate condition evidently arising at several different points for each sorus, shortly before the sorus is formed. BLACKMAN and FRASER also observed that the short cycle forms *Puccinia Adoxae* Hedw.f. and *Uromyces Scillarum* (Grev.) Wint. had a binucleate rather than a uninucleate general vegetative mycelium, and suggested that it is "probable that in these two forms the conjugate condition is produced soon after infection by nuclear migration, or by cell fusion, between vegetative cells." OLIVE (**20**) discussed and figured sexual fusions near the base of the telium in a short cycle form, *Puccinia transformans* Ellis and Ev. Dealing with North American rusts, OLIVE (**19**) also reported that differing conditions as to the sporophytic and gametophytic generations occurred with certain short cycle *Puccinia* forms; while *Uromyces Rudbeckiae* Arth. and Holw. showed the anomalous extreme of possessing uninucleate cells through all the mycelium and sorus, even including the teliospores.

This phenomenon he was not able to explain fully. Other papers to be noted are those by WERTH and LUDWIGS (**26**), HOFFMAN (**16**), and MOREAU (**18**). A considerable summary of recent cytological work is presented by RAMSBOTTOM (**22**).

From this work it appears that the duration of the binucleate stage varies in different species of short cycle rusts, being brief, extended, or intermediate. Fusions between cells initiate this binucleate condition. Some life history problems, including the relative importance and relation of cell and nuclear fusions, some relations in the formation of pycnia in short cycle forms, the presence of perennial mycelium, etc., appear not to have been fully determined.

TABLE I

HOST RELATIONSHIPS OF SHORT CYCLE *Uromyces*

Host	Species of Uromyces	Distribution	Mycelium	Common condition of germination	Pycnia
Liliaceae					
Erythronium...	U. heterodermus	Rockies	Local or rather diffuse	Micro	Present
Cassiaceae					
Bauhinia.....	U. bauhiniicola	S.W. Mexico	Rather diffuse	Micro	Present
Bauhinia.....	U. jamaicensis	Mexico; West Indies	Local	Micro	Present
Fabaceae					
Psoralea.....	U. abbreviatus	Pacific Coast	Local, becoming rather diffuse	Micro	Present
Euphorbiaceae					
Chamaesyce, Tithymalus	U. Tranzschelii	Western N.A.	Diffuse	Micro	Present
Primulaceae					
Primula.....	U. nevadensis	Western N.A.	Local or rather diffuse	Micro	Not known
Myrsinaceae					
Myrsines.....	U. Myrsines	Costa Rica; S.A.	Local	Micro	Not known
Carduaceae					
Solidago.....	U. Solidaginis	W.N.A.; Europe	Local	Micro	Not known
Anaphalis.....	U. amoenus	Western N.A.	Local	Micro	Not known
Rudbeckia.....	U. Rudbeckiae	Central N.A.	Local	Lepto	Not known
Bidens.....	U. Bidentis	Porto Rico; S.A.	Local	Lepto	Not known

Hosts

The range of hosts attacked by these North American short cycle species of *Uromyces* embraces both the monocotyledons and dicotyledons. The situation is shown in table I.

Foreign species of short cycle *Uromyces* fill in several families not represented here. The wide range of hosts attacked indicates that these rusts do not form a restricted group; one might expect

to find affiliations with other forms of rusts upon the same or similar hosts through the various families, and such is the case. Under the species *U. heterodermus* a considerable comparison with rusts from related hosts is made, suggesting that certain groups of hosts appear to harbor rusts characterized by various definite morphological characters.

The geographical distribution of North American short cycle species of *Uromyces* would indicate further that the mountainous and more tropical regions furnish the most favorable location for these forms. Only *U. Rudbeckiae* has a comparatively wide range, a range including the plains area.

Whether or not it is more than a coincidence that the absence of pycnia and the occurrence of lepto-germination are found on hosts higher in the evolutionary scale, the writer is not prepared to say.

Taxonomic

With the progress of critical studies of North American rusts, other short cycle forms will undoubtedly be separated out, and further evidence secured as to the fixity and definiteness of the life cycle in certain of these rusts. *Uromyces heterodermus*, for example, was long placed with *U. Erythronii*, a correlated form. It was found also that *U. Bidentis* was a short cycle form which resembled *U. bidenticola* (P. Henn.) Arth. so far as characters of teliospores are concerned. It is no doubt true that other short cycle forms have been collected and placed with correlated long cycle forms, although cultures are needed to determine the life cycle in certain cases.

The 11 species of *Uromyces* considered have several points of similarity, one of which is the fact that all possess teliospores with apices more or less thickened. In none of the species were paraphyses, stromata, isolated peridial cells, or other accompanying structures found in the telia.

Key

Teliospores verrucose.
- Teliospores long, up to 43–47 μ
  - Wall up to 1.5 μ thick..... 1. *U. heterodermus*
  - Wall up to 2.5 μ thick..... 6. *U. nevadensis*
- Teliospores short, up to 30 μ..... 5. *U. Tranzschelii*

Teliospores reticulate.
  Spores 14–21 by 18–26 μ ..... 2. *U. bauhiniicola*
  Spores 12–17 by 16–23 μ ..... 3. *U. jamaicensis*
Teliospores smooth.
  Wall thin, 1–1.5 μ.
    Spores narrow, 11–17 μ wide.
      Spores 27–39 μ long ..... 7. *U. Myrsines*
      Spores 19–32 μ long ..... 10. *U. Rudbeckiae*
    Spores broad, 15–28 μ wide ..... 11. *U. Bidentis*
  Wall thick, 1.5–3 μ.
    Apex thickened 6–12 μ ..... 8. *U. Solidaginis*
    Apex thickened 3–7 μ.
      Spores 27–40 μ long ..... 4. *U. abbreviatus*
      Spores 19–30 μ long ..... 9. *U. amoenus*

1. UROMYCES HETERODERMUS Sydow, Ann. Myc. 4:29. 1906.

O. Pycnia amphigenous, not uncommon, gregarious in loose groups with the telia, 0.5–1.5 mm. across, inconspicuous, subepidermal, dark golden-brown, flattened globoid, 100–185 μ in diameter by 65–130 μ in height; ostiolar filaments few, loose, up to 65 μ long.

III. Telia amphigenous, numerous, scattered or in small groups, sometimes upon inconspicuous spots, roundish or oval, 0.3–2 mm. across, rather early naked, pulverulent, dark cinnamon-brown, surrounding epidermis noticeable; teliospores ellipsoid or broadly ellipsoid, 19–26 × 26–43 μ, rounded above, rounded or slightly narrowed below; wall dark golden-brown, 1.5 μ thick, thickened at the apex with a distinct hyaline papilla, 3–6 μ, coarsely verrucose above, with the markings often in longitudinal ridges, smoother below; pedicel hyaline, fragile, short.

ON LILIACEAE: *Erythronium grandiflorum* Pursh, Colorado, Montana, Utah, Washington, British Columbia; *E. montanum* S. Wats., Washington; *E. obtusatum* Goodding, Wyoming; *E. parviflorum* (Wats.) Goodding (*E. grandiflorum parviflorum* S. Wats.), Colorado, Montana, Oregon, Utah, Washington, Wyoming.

TYPE LOCALITY: Wasatch Mountains, Utah, on *Erythronium parviflorum*.

DISTRIBUTION: Rocky Mountain region from Colorado and Utah northward, and to the coast in Oregon.

EXSICCATI: Barth., Fungi Columb. *4694;* Barth., N.Am. Ured. *789, 1592, 1692;* Garrett, Fungi Utah. *118;* Ellis and Ev., Fungi Columb. *750*.

LITERATURE: SYDOW, Monog. Ured. 2:270. 1910; SACCARDO, Syll. Fung. 21:579. 1912.

This rust, previous to SYDOW's description in 1906, passed as *U. Erythronii* (DC.) Pass., a related European species possessing aecia. Thus ELLIS and EVERHART'S Fungi Columbiani *750* was issued as *U. Erythronii.* The host of this collection is undoubtedly *Erythronium parviflorum;* earlier collections of this host were frequently considered, as in this case, to be *E. grandiflorum.*

This rust occurs upon the species of *Erythronium* found in the western part of North America. According to ENGLER (ENGLER and PRANTL, Nat. Pflanz. 2^5:60. 1888) species of *Erythronium* occur particularly in North America. He places the following genera together to constitute the section Liloideae-Tulipeae: *Lilium, Fritillaria, Erythronium, Tulipa, Lloydia,* and *Calochortus.* Several rusts occur upon these genera of hosts. For the sake of comparison, all such rusts are tabulated. To avoid a personal factor, the data are largely from the SYDOWS' *Monograph,* and any supplementary data obtained are added in brackets. Parentheses indicate a rather free translation. Some data are taken from a paper by REES (Amer. Jour. Bot. 4:368–373. 1917), who also presents drawings which support the contention that the rusts on these hosts possess rather unusual morphological similarities.

Table II shows many points of similarity in these rusts. It is to be noted that practically all possess amphigenous, rounded or minute, pulverulent sori, with spores broadly ellipsoid, rather similar as to size, with the wall usually moderately thick, apex somewhat thickened with a papilla, pedicel hyaline and short; and especially, all possess, in a striking manner, surface markings usually striate or verrucose and arranged in rows. This unanimity in morphological characters would indicate that a closely and definitely related group of rusts occurs upon these related hosts. Correlations, more or less perfect, obtain throughout this group of rusts upon the Liloideae-Tulipeae, and are found to extend further through the Liliaceae. Figs. 1–6 illustrate, for comparison, the teliospores of three of these rusts.

2. UROMYCES BAUHINIICOLA Arth. BOT. GAZ. **39**:389. 1905.—*Telospora Bauhiniicola* Arth., Result. Sci. Congr. Bot. Vienne 346. 1906.

TABLE II

Characters of rusts of Liloideae-Tulipeae

Rust	Host genus	Source of data	Life cycle	Geographical distribution	Leaf surface bearing telia	Shape of telia	Character of telia
Uromyces heterodermus Sydow	Erythronium	This paper	O, III	N.A.	Amphigenous	Round or oval	Pulverulent
Uromyces aecidiiformis (Strauss) Rees	(Fritillaria?)	Rees	O, I, III	Europe	Amphigenous	Elongated	Pulverulent
Uromyces Lilii (Link) Fuckel	Lilium	Sydow	O, I, III	(N.A.?), Europe	Amphigenous	Round or oblong	Pulverulent
Uromyces Erythronii (DC.) Pass.	Erythronium	Sydow	O, I, III	Europe	Amphigenous	Roundish	Pulverulent
Uromyces mogianensis Bubak	Fritillaria	Sydow	O, I*, III	Asia, Europe, Africa	Amphigenous	Round or oblong	Pulverulent
Uromyces Miurae Sydow	Fritillaria	Rees	(O, I)? III	Japan, N.A.	Amphigenous	Round or ellipsoid	Pulverulent
Uromyces Fritillaria (Schlechtd.) Thüm	Fritillaria	Rees	O, I, III	Europe	Amphigenous	Elliptical	Pulverulent
Uromyces Tulipae Dietel	Tulipa	Sydow	I, III	Japan	Amphigenous	Minute	Pulverulent
Uromyces japonicus Sydow	Lilium	Sydow	II, III	Japan	(Amphigenous)	Round	Pulverulent
Uromyces Holwayi Lagerh	Lilium	Sydow	[O, I], II, III	N.A.	Amphigenous	Minute round	Pulverulent
Nigredo Lilii (Clint.) Arth	Lilium	Flora	O, I, II, III	N.A.	Amphigenous	.....	Pulvinate?
Puccinia Calochorti Peck	Calochortus	Sydow	(O), I, III	N.A.	Chiefly hypophyllous	Round	Pulverulent
Puccinia Prostii Mong	Tulipa	Sydow	III	Europe	Amphigenous	Oblong	[Pulverulent]
Puccinia Tulipae Schroet	Tulipa	Sydow	III	Europe	Chiefly hypophyllous	Minute	.....

Rust	General shape of teliospores	Size of teliospores	Wall in μ	Apex in μ	Apex character	Sculpturing	Pedicel
Uromyces heterodermus Sydow	Broad, ellipsoid	19–26×26–43	1–5	3–6	Hyaline papilla	Coarsely verrucose in rows	Hyaline, short
Uromyces aecidiiformus (Strauss) Rees	Broad, ellipsoid	23–27×31–35	About 3	[to 5]	Hyaline apiculus	Rugose in rows	Hyaline, short
Uromyces Lilii (Link) Fuckel	Broad, ellipsoid	22–30×28–44	2–3.5	[to 5]	Hyaline papilla	Rugose or verrucose in rows	Hyaline, short
Uromyces Erythronii (DC.) Pass.	(Broad, ellipsoid)	16–25×22–42	1.5–2	[to 6]	Hyaline papilla	Anastomose in rows	Hyaline, short
Uromyces mogianensis Bubak	Subglobose	26–32×26–38	3–6	.....	Hyaline papilla	Verrucose in rows	Hyaline, deciduous
Uromyces Miurae Sydow	Ellipsoid	14–23×24–35	1.5–2	to 5	Hyaline apiculus	Verrucose in rows	Hyaline, short
Uromyces Fritillaria (Schlechtd.) Thüm	Broad, ellipsoid	23–31×31–42	.....	to 5	Hyaline apiculus	Rugose in rows	Hyaline, short
Uromyces Tulipae Dietel	Broad, ellipsoid	20–28×26–40	.....	.....	Hyaline papilla	Reticulate-striolate	Hyaline, short
Uromyces japonicus Sydow	Ovate	20–28×28–46	1.5	.....	Hyaline papilla	Verrucose in rows	Hyaline, deciduous
Uromyces Holwayi Lagerh	Broad, ellipsoid	20–28×24–42	[2–2.5]	[5–7]	Hyaline papilla	Verrucose in rows	Hyaline, deciduous
Nigredo Lilii (Clint.) Arth	Broad, ellipsoid	18–25×29–39	2–2.5	5–7	Apiculus	Rugose in ridges	Hyaline, short
Puccinia Calochorti Peck	[Broad], ellipsoid	22–30×33–40	[1.5–2.5]	[3–5]	.....	Verrucose	Hyaline (short)
Puccinia Prostii Mong	(Broad), ellipsoid	34–40×54–66	[2–3]	[3]	(Aculeate)	Aculeate, strongly	[Hyaline] (short)
Puccinia Tulipae Schroet	Broad, ellipsoid	21–32×30–44	.....	.....	.....	Verrucose	Hyaline, short

* Bubak says O with III.

O. Pycnia epiphyllous, few, gregarious in small groups, usually opposite the telia, punctiform, subepidermal, brownish, flattened globose, 60–130 μ in diameter by 30–65 μ in. height; ostiolar filaments compact, short.

III. Telia at first hypophyllous, becoming also somewhat epiphyllous, numerous, scattered or in small groups, roundish, small, 0.2–1 mm. across, early naked, pulverulent, chocolate-brown, surrounding epidermis inconspicuous; teliospores globoid or broadly ellipsoid, 14–21 by 18–26 μ, rounded at the ends; wall cinnamon or chestnut brown, thick, 2.5–4 μ, apex thicker, 4–7 μ, with a paler, broad umbo, finely reticulated; pedicel pale or colorless, often roughened below, rather fragile but sometimes two or three times as long as the spore.

On Cassiaceae: *Bauhinia Pringlei* S. Wats., Jalisco; *Bauhinia* sp., Guerrero.

Type locality: Guadalajara, Mexico, on *Bauhinia Pringlei.*

Distribution: Known only from Southwest Central Mexico.

Illustration: Ark. Bot. Stockh. 4: *pl. 1. fig. 9.*

Exsiccati: Barth., N.Am. Ured. *286.*

Literature: Vestergren, Ark. Bot. Stockh. 4:28–29. 1905; Sydow, Monog. Ured. 2:80, 81. 1909; Saccardo, Syll. Fung. 21:550–551. 1912.

3. Uromyces jamaicensis Vesterg. Ark. Bot. Stockh. 4:33. 1905.

O. Pycnia chiefly epiphyllous, gregarious in small groups with the telia, subepidermal, brownish, flattened, 60–100 μ in diameter by 45–70 μ in height; ostiolar filaments compact, hardly extending beyond the ostioles.

III. Telia amphigenous, numerous, gregarious in small groups or occurring singly, sometimes on small yellowish spots, roundish, small, 0.1–1 mm. across, early naked, pulverulent, chestnut-brown, surrounding epidermis noticeable; teliospores globoid, broadly ellipsoid or obovoid, 12–17 × 16–23 μ, rounded or slightly narrowed at the ends; wall cinnamon-brown, 1.5–2 μ (sometimes up to 3.5 μ) thick, thicker at the apex, up to 5 μ, with a lighter crater or cap-shaped crown, closely and finely reticulate, appearing verrucose under the lower powers of the microscope; pedicel pale, fragile, 4–15 μ long.

ON CASSIACEAE: *Bauhinia divaricata* L., Cuba, Guanajuoto; *B. Pauletia* Pers., Porto Rico; *B. porrecta* Sw., Jamaica.

TYPE LOCALITY: Constant Spring, Jamaica, on *Bauhinia* sp.

DISTRIBUTION: Mexico and the West Indies.

ILLUSTRATION: Ark. Bot. Stockh. **4**: *pl. 2. fig. 14.*

LITERATURE: SYDOW, Monog. Ured. **2**:84. 1909; SACCARDO, Syll. Fung. **21**:552–553. 1912.

This species may perhaps be distinguished from the preceding by the somewhat reduced length and breadth of the teliospores, the wall thickness often being less also. The differences described by VESTERGREN (*loc. cit.*) have not been found to hold entirely throughout the collections at the Arthur herbarium. Some differences, however, are still to be found between the two species of rust, and they are maintained as separate species, at least pending further collections.

VESTERGREN'S supposition that *Uromyces jamaicensis* is a micro-*Uromyces* has been corroborated by the discovery of pycnia associated with telia upon a Cuban specimen of *Bauhinia divaricata*. The specimen upon *B. porrecta* collected by THAXTER has not been seen, but VESTERGREN'S type collection has been examined.

VESTERGREN separated 17 species of *Uromyces* upon the host *Bauhinia*, for none of which aecia are known. Evident similarities are shown between the species as he described and figured them. *Uromyces* only are known to occur upon *Bauhinia*. Many species of *Bauhinia* occur in the tropics; related genera, as shown by ENGLER and PRANTL'S classification, are chiefly genera upon which rusts have not yet been found. The reticulate nature of the sculpturing upon the surface of the teliospores of these two species is minute, but evident under higher microscopic power. Figs. 7–10 illustrate the two species.

4. UROMYCES ABBREVIATUS Arth. Bull. Torr. Bot. Club **42**:587. 1915.

O. Pycnia hypophyllous; scattered among the telia, inconspicuous, subepidermal, deep seated, dark honey-yellow, globose or flattened globose, 115–200 μ in diameter by 95–140 μ in height; ostiolar filaments dense, often falling away, up to 60 μ in length.

III. Telia hypophyllous, rarely also epiphyllous, densely clustered, becoming scattered over considerable areas, roundish,

0.2–0.7 mm. across, early naked, pulverulent, chocolate-brown, surrounding epidermis at first evident, later often hidden by the loose spores; teliospores ellipsoid or irregularly obovoid, 21–26 × 27–40 μ (sometimes variable in size, and larger), rounded above, rounded or narrowed below; wall chestnut-brown, 1.5–3 μ thick, apex 3–5 μ thick, often with a slight umbo over the pore, smooth; pedicel colorless, delicate, fugacious, half as long as the spore or less.

On Fabaceae: *Psoralea physodes* Dougl., California, Washington, British Columbia; *P. Purshii* Vail, Nevada.

Type locality: Winnemucca, Nevada, on *Psoralea Purshii*.

Distribution: Pacific Coast region, west of the mountains, from British Columbia to California.

Exsiccati: Barth., N.Am. Ured. *1582;* D. Griff., W.Am. Fungi *390;* Barth., Fungi Columb. *4884*.

The type of this species is Griffith's West American Fungi *390*, which was issued as *Uromyces Psoraleae* Peck. *U. Psoraleae* possesses aecia, however. *U. abbreviatus*, while without aecia, and possessing pycnia with the telia, resembles *U. Psoraleae* in the telial stage, as indicated by Arthur in the notes with the original description. While *U. Psoraleae* extends to the Pacific Coast, it is more common in the Rocky Mountain region, and extends over the plains to the east of the mountains. *U. abbreviatus*, so far as known, is limited to the region west of the Rockies.

There is an unconnected *Aecidium* (*Aecidium onobrychidis* Burrill, Bull. Ill. State Lab. Nat. Hist. 2:225. 1885) upon *Psoralea Onobrychis*, represented as far as known by the one collection by Seymour in Illinois, and distributed by Ellis and Everhart as North American Fungi *1826*. No other species of rust are reported for the genus *Psoralea*, and these species are only known in North America. Related hosts, as given by Engler and Prantl, except for the genus *Indigofera* in an adjoining section, are scarcely known to be attacked by rusts; no closely related rusts are evident upon related hosts.

While the type collection is from an altitude of about 5000 ft., other collections in the Arthur herbarium are from nearer the coast, at much less altitude, extending almost down to sea-level.

5. UROMYCES TRANZSCHELII Sydow; Tranzschel, Ann. Myc. 8:20. 1910.

O. Pycnia hypophyllous, scattered among the telia, or in groups, noticeable, subepidermal, dark yellow, globoid or flask-shaped, 100–145 μ in diameter by 75–130 μ in height; ostiolar filaments dense, agglutinated into a truncate column, 50–80 μ in height, 50–70 μ in diameter at the ostiole.

III. Telia hypophyllous, occasionally sparingly epiphyllous, numerous, evenly scattered over large areas, or sometimes in groups around the pycnia, roundish, 0.2–0.6 mm. across, early naked by a central pore, pulverulent, chestnut-brown, surrounding epidermis crateriform, conspicuous; teliospores globoid or ellipsoid, 15–22 × 19–30 μ, rounded at the ends, wall cinnamon-brown, 1.5–2.5 μ thick, apex 3–5 μ thick with a low, sub-hyaline apiculus, minutely verrucose, the markings often in irregular longitudinal lines; pedicel colorless, deciduous.

ON EUPHORBIACEAE: *Chamaesyce serpens* (H.B.K.) Small (*Euphorbia serpens* H.B.K.), California; *Tithymalus montanus* (Engelm.) Small (*Euphorbia montana* Engelm.), Colorado, New Mexico, Utah, Wyoming; *T. robustus* (Engelm.) Small (*Euphorbia montana robusta* Engelm.), Colorado, Utah, Wyoming; *Tithymalus* sp. (*Euphorbia Palmeri* Engelm.), Lower California.

TYPE LOCALITY: Colorado, on *Euphorbia montana*.

DISTRIBUTION: From Wyoming to New Mexico, California, and Lower California.

EXSICCATI: Barth., N.Am. Ured. *499;* Ellis and Ev., Fungi Columb. *1069;* Ellis and Ev., N.Am. Fungi *2230;* Garrett, Fungi Utah. *97*.

LITERATURE: TRANZSCHEL, Ann. Myc. **8**:1–35. 1910; SYDOW, Monog. Ured. **2**:171–172. 1910; SACCARDO, Syll. Fung. **21**:560–561. 1912; DIETEL, Hedw. **28**:185–187. 1889; ARTHUR, Bull. Torr. Bot. Club **45**:152. 1918.

This rust passed as *Uromyces scutellatus* (Schrank.) Lev., a European species, until SYDOW'S description in 1910. TRANZSCHEL pointed out that *U. Tranzschelii* is similar to *U. monspessulanus* Tranz.; indeed, other similarities to various Euphorbiaceous rusts are evident. In his study of the autoecious rusts upon *Euphorbia*, TRANZSCHEL stated that most European autoecious species with telia from diffused mycelium had passed as two species, *Uromyces scutellatus* or *U. excavatus;* he divided such forms into some 12 species, and found a total of 27 autoecious species of *Uromyces*

upon hosts belonging to the various sections of the genus *Euphorbia*. That these species are related is evidenced by the fact that many had passed under one name; furthermore, many similarities are to be noted from TRANZSCHEL'S descriptions. For example, all but one species are listed as having verrucose or striolate teliospore walls. A table showing characters in a manner similar to those tabulated under *U. heterodermus* would be illuminating as indicating relationships between *U. Tranzschelii* and other species of rust upon related hosts. The writer considered it sufficient, however, to call attention to TRANZSCHEL'S work as indicating relationships. Certain heteroecious species with aecia upon *Euphorbia* likewise show resemblances to *U. Tranzschelii*.

Both ELLIS and EVERHART'S Fungi Columbiani *1069* and North American Fungi *2230* were issued as *U. scutellatus*, while GARRETT'S Fungi Utahensis *97* was issued as *U. andinus* P. Magn., a related South American rust.

The range of *U. Tranzschelii* begins at about the western limit of the range of the related species *U. proeminens* (DC.) Pass., and continues westward to the Pacific Coast. Range conditions comparable with those of *U. abbreviatus* are thus shown, and neither of these short cycle forms necessarily occurs at high altitudes.

TRANZSCHEL (*loc. cit.*, p. 20) considered the rust upon *Euphorbia Palmeri* to be different, apparently another species. The specimen studied by the writer is not considered different from other specimens of *U. Tranzschelii*.

DIETEL (*loc. cit.*) commented upon ELLIS and EVERHART'S North American Fungi *2230*, especially concerning the relationship of an *Aecidium* upon the same host distributed as no. *2215* of the same exsiccati. It is true that *Aecidium Tithymali* Arth. occurs upon the same hosts, sometimes upon the same leaves, as *Uromyces Tranzschelii*. The situation in regard to this *Aecidium Tithymali* is uncertain. Germination tests show that it is a true *Aecidium* and not an *Endophyllum*. Its alternate host, however, has not been found. ARTHUR (*loc. cit.*) has discussed this *Aecidium* and its possible relation to *U. Tranzschelii*.

6. UROMYCES NEVADENSIS Hark. Bull. Calif. Acad. Sci. 1:36. 1884.—*Caeomurus nevadensis* Kuntze, Rev. Gen. 3^3:450. 1898.

O. Pycnia unknown.

III. Telia amphigenous, circinating in groups 2–5 mm. across, or somewhat scattered, round or oval, 0.2–1.0 mm. across, early naked, pulvinate, becoming somewhat pulverulent, chestnut-brown, ruptured epidermis conspicuous; teliospores oblong, oblong-obovoid, or ellipsoid, 19–27×29–47 μ, rounded at the apex, rounded or narrowed toward the base; wall cinnamon-brown, lighter or colorless at the apex, moderately thick, 1.5–2.5 μ, thickened at the apex, 5–7 μ, moderately and rather finely verrucose; pedicel colorless, fragile.

On Primulaceae: *Primula suffrutescens* Gray, Nevada.
Type locality: Lake Tahoe, Nevada, on *P. suffrutescens*.
Distribution: Known only from the type locality.
Literature: Magnus, Ber. Deutsch. Bot. Gesells. **18**:451–459. 1900.
Illustration: Magnus, *loc. cit. pl. 16. figs. 16–19.*

The writer is considerably indebted to the members of the botanical staff at the Purdue Station for the preceding. Arthur in a letter states that "a careful study of this species seems to leave little doubt that it is a distinctly American species and a short cycle one. This was the conclusion reached by Magnus in 1900." The one collection known was made by Harkness, and a specimen has been studied by the writer.

7. Uromyces Myrsines Diet. Hedwigia **36**:26. 1897.

O. Pycnia unknown.

III. Telia hypophyllous, crowded upon reddish or brownish spots 2–10 mm. in diameter, margin of spots usually elevated, roundish, 0.1–0.2 mm. in diameter, often confluent, early naked, pulvinate, light chocolate-brown, ruptured epidermis inconspicuous; teliospores oblong or oblong-ellipsoid, 13–16×27–39 μ, rounded or acute above, narrow below; wall pale golden-brown, rather thin, 1–1.5 μ, thickened at the apex, 3–8 μ, smooth; pedicel colorless, short.

On Myrsinaceae: *Ardisia compressa* H.B.K., Costa Rica.
Type locality: Rio de Janeiro, Brazil, on *Myrsine* sp.
Distribution: Costa Rica; also in South America.
Literature: Arthur, Mycologia **10**:124, 1918; Sydow, Monog. Ured. **2**:46. 1909.

This rust was known only from South America before its discovery by HOLWAY in one locality in Costa Rica. South American specimens have been distributed by E. ULE, Herbarium Brasiliense no. *2136*. ARTHUR suggests that *U. marginatus* Bomm. and Rouss may be a synonym. SYDOW gives *U. Rhapaneae* Henn. and *U. Usterianus* Diet. as synonyms. While SYDOW was probably right, it has been impossible to examine specimens of these two collections.

8. UROMYCES SOLIDAGINIS (Sommerf.) Niessl, Verh. Natur. Ver. Brunn **10**:163. 1872.—*Caeoma Solidaginis* Sommerf. Suppl. Fl. Lapp. 234. 1826; *Caeomurus Solidaginia* Kuntze, Rev. Gen. 3^3:450. 1898; *Telospora Solidaginia* Arth., Result. Sci. Congr. Bot. Vienne 346. 1906.

O. Pycnia not found; probably wanting.

III. Telia hypophyllous, sometimes also petiolicolous or caulicolous, crowded and often confluent in orbicular groups upon the leaves, or in elongated groups upon the petioles or stems, 2–10 mm. across, upon yellowish spots, roundish, small, 0.3–0.7 mm. across, early naked, compact, pulvinate, chocolate-brown, surrounding epidermis noticeable; teliospores obovate or ellipsoid, 17–25× 24–33 μ, narrowed or rounded at the ends; wall chestnut-brown, 1.5–3 μ thick, much thicker at the apex, 6–12 μ, smooth; pedicel nearly colorless, about as long as the spore.

ON CARDUACEAE: *Solidago polyphylla* Rydb., Colorado; *S. serotina* Ait., Montana, Washington, Wyoming.

TYPE LOCALITY: Nordland, Sweden, on *Solidago virgaurea*.

DISTRIBUTION: Colorado to Montana and Washington, also in Europe and Asia.

ILLUSTRATIONS: Archiv. Naturw. Land. Bohmen **13**: *fig. 12;* Beitr. Krypt. Schweiz 2^2: *fig. 44*.

EXSICCATI: D. Griff., W.Am. Fungi *361*; Ellis and Ev., N.Am. Fungi *2883*.

LITERATURE: COOKE, Grev. **5**:152. 1877; WINTER, in Rab. Krypt. Fl. 1^1:141. 1881; SACCARDO, Syll. Fung. **7**:566. 1888; FISCHER, Beitr. Krypt. Schweiz 2^2:59, 543. 1904; FISCHER, Ber. Schw. Bot. Gesells. **15**:(1–2). 1905; HARIOT, Les Ured. 216. 1908; SYDOW, Monog. Ured. **2**:10. 1909.

This is the one species of *Uromyces* included in this paper which is not endemic to the Americas. FISCHER (1905) reported cultures of this rust. He also (1898) pointed out the correlation between this species and *U. Junci* (Desmaz.) Tulasne, which bears aecia

upon hosts related to *Solidago*. The range of both *U. Solidaginis* and *U. Junci* in North America is similar, both occurring in the western part.

Uromyces Junci-effusi Sydow resembles *U. Solidaginis* in the telial stage; the aecial connection is not known for this form. Curiously not *Puccinia Solidaginis* Peck, but *P. Asteris* Duby (both are short-cycled) shows a correlation with *Uromyces Solidaginis*. Of the short cycle species of *Puccinia* upon *Solidago* in America, one, *P. Virgauriae* (DC.) Lib., is more eastern, possesses stromata, and has thin-walled teliospores. *P. Solidaginis*, although a western form, has very large teliospores. *P. Asteris*, however, is very similar to *Uromyces Solidaginis* in gross and microscopic characters, except in the possession of 2-celled teliospores. *Puccinia Asteris* is a more common rust, and while rare west of the Rockies, is found over most of North America. Figs. 19–22 illustrate *U. Solidaginis* from America and Europe and *P. Asteris*.

COOKE (*loc. cit.*) reported *Uromyces Solidaginis* from Maine. Collections from Eastern North America are not at hand; further doubt may be attached to COOKE's reported collection from the fact that he states that the spores are reticulated. GRIFFITH's West American Fungi *361*, although issued as *Puccinia Solidaginis*, is in reality *Uromyces Solidaginis*.

9. UROMYCES AMOENUS Sydow, Ann. Myc. 4:28. 1906.

O. Pycnia unknown.

III. Telia hypophyllous, densely grouped and often confluent on circular purplish spots, 2–8 mm. across, the margin of the spots yellow, roundish, small, 0.2–0.7 mm. across, early naked, compact pulvinate, dark chestnut-brown, covered by the tomentose pubescence of the host, ruptured epidermis inconspicuous; teliospores globoid, obovoid, or ellipsoid, 16–23 × 20–30 μ, usually rounded above and narrowed below; wall dark golden-brown or cinnamon-brown, moderately thick, 1.5–2.5 μ, apex thicker, 4–7 μ, smooth; pedicel pale yellowish, up to the length of the spore.

ON CARDUACEAE: *Anaphalis margaritacea occidentalis* Greene, Oregon; *A. subalpina* (A. Gray) Rydb. (*A. margaritacea subalpina* A. Gray), Idaho, Oregon, Washington, Wyoming, British Columbia.

TYPE LOCALITY: Washington, on "*Gnaphalium* (*Anaphalis*) *margaritacea*."

DISTRIBUTION: Wyoming to British Columbia and Oregon.

EXSICCATI: Ellis and Ev., Fungi Columbiani *1795;* Barth., N.Am. Ured. *1385, 1584.*

LITERATURE: SYDOW, Monog. Ured. 2:4. 1909; SACCARDO, Syll. Fung. 21:570. 1912.

Several collections of this rust are in the Arthur herbarium. Although the hosts of some collections are labeled *Anaphalis margaritacea,* it would appear that the name *A. subalpina* should be used for almost all collections in hand (compare COULTER and NELSON, New Manual of the Botany of the Central Rocky Mountains, p. 537).

ELLIS and EVERHART'S Fungi Columbiani *1795* was issued as *Uromyces Gnaphalii* Ellis and Ev., but is *U. amoenus. U. Gnaphalii* has been found to be a synonym of *U. intricatus* Cooke.

10. UROMYCES RUDBECKIAE Arth. and Holw. Bull. Iowa Agric. Coll. 1884. 154. 1885.—*Caeomurus Rudbeckiae* Kuntze, Rev. Gen. 3^3:450. 1898; *Telospora Rudbeckiae* Arth., Result. Sci. Congr. Bot. Vienne 346. 1906.

O. Pycnia unknown.

III. Telia hypophyllous, occasionally also epiphyllous, densely gregarious upon brownish spots, paler below, 1–10 mm. across, rather circinate, small, 0.2–0.8 mm. in diameter, early naked, compact, pulvinate, cinnamon-brown, soon cinereous from germination, surrounding epidermis not noticeable; teliospores ellipsoid, obovoid, or pyriform, 11–17×19–32 μ, rounded, acute, or obtuse at the apex, narrowed below; wall yellowish or very pale chestnut-brown, thin, 1 μ, apex thicker, 5–8 μ, smooth; pedicel hyaline, twice as long as the spore or less.

ON CARDUACEAE: *Rudbeckia laciniata* L. (*R. ampla* A. Nels.), Colorado, Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Montana, Nebraska, New Mexico, North Dakota, Pennsylvania, Wisconsin, Wyoming, Ontario; *Rudbeckia* sp., Texas.

TYPE LOCALITY: Decorah, Iowa, on *Rudbeckia laciniata.*

DISTRIBUTION: Ontario and Pennsylvania to Montana and Texas.

ILLUSTRATION: Arth. and Holw. Ured. Exs. Ic. 1: *pl. 1. fig. 1.*

EXSICCATI: Arth. and Holway, Ured. Exs. Ic. 1: Barth., Fungi Columb. *4394;* Barth., N.Am. Ured. *299, 1099, 1397;* Brenckle, Fungi Dak. *274;* Ellis and Ev., Fungi Columb. *2097;* Ellis, N.Am. Fungi *1439;* Rab.-Wint., Fungi Eur. *3412;* Sydow, Ured. *1305, 1962.*

LITERATURE: BURRILL, Bull. Ill. State Lab. Nat. Hist. 2:163. 1885; SACCARDO, Syll. Fung. 7:581. 1888; ARTHUR and HOLWAY, Bull. Lab. Nat. Hist. State Univ. Iowa 3:44. 1895; SYDOW, Monog. Ured. 2:7–8. 1909.

Uromyces Rudbeckiae has been collected more frequently than any North American species of the group. Its range embodies the greater part of the plains area, and extends to the Rocky Mountains.

DIETEL (Ann. Myc. 8:305. 1910) considered *Uromyces Komerovii* Bubak on *Solidago Virgaurea* identical with *U. Rudbeckiae*. No specimens of the former have been seen, although a collection on *Solidago Virgaurea* in the herbarium has not been found to differ from *U. Solidaginis*.

The only other rust found upon *Rudbeckia* is *Aecidium Compositarum* Auct., recently found to belong with *U. perigynius* Halsted (Mycol. 9:307), a connection suspected from the fact that the telial stage of *U. Rudbeckiae* is similar to the telial stage of *U. perigynius*. A type of correlation which has frequently been of service in indicating alternate stages of heteroecious rusts is thus evidenced. The cytological work upon this species is noted earlier in this paper.

11. UROMYCES BIDENTIS Lagerh. Bull. Soc. Myc. Fr. 11:213, 1895.—*Caeomurus Bidentis* Kuntze, Rev. Gen. 3^3:449. 1898; *Uromyces densus* Arth. Mycologia 7:196. 1915.

O. Pycnia unknown.

III. Telia hypophyllous, numerous, in small circinating groups on roundish, discolored spots, 1–4 mm. across, sometimes confluent, roundish or oval, 0.1–1 mm. across, the central sorus larger, surrounded by smaller ones, early naked, compact, pulvinate, dull cinnamon-brown, becoming waxy-cinereous from germination, surrounding epidermis inconspicuous; teliospores obovoid or oblong, 15–28×30–45 μ, rounded or narrowed above, narrowed below; wall pale golden or cinnamon-brown, thin, 1 μ, thicker above, 4–9 μ, smooth; pedicel hyaline, once or twice the length of the spore or less.

ON CARDUACEAE: *Bidens leucantha* (L) Willd., Porto Rico; *B. pilosa* L., Porto Rico; *Bidens* sp., Costa Rica.

TYPE LOCALITY: Ecuador, South America, on *Bidens andicola*.

DISTRIBUTION: Porto Rico and Central America; also in South America.

The SYDOWS (Monog. Ured. 1:3. 1909) misapplied LAGERHEIM'S name to the species with uredinia, now called *Uromyces bidenticola* (P. Henn.) Arth. The situation in regard to these two rusts is discussed by ARTHUR (Mycologia 9:71. 1917), and he also (Mycologia 10:127. 1918) suggests that it is possible that a fixity of life cycle may not occur in these *Bidens* rusts. *U. Bidentis* is correlated with *U. Bidenticola*, differing only in the life cycle and in the characters of the telia, which are coalescent and thickened into cushions in *U. Bidentis*. Specimens are at hand also from South America; LAGERHEIM'S collection from the type locality has been examined.

Puccinia Bidentis Diet. and Holw., BOT. GAZ. 24:32. 1897, collected by HOLWAY in Mexico, apparently is not a correlated species.

EXCLUDED SPECIES

UROMYCES HYALINUS Peck, BOT. GAZ. 3:34. 1878.—*U. Sophorae* Peck, Bull. Torr. Bot. Club 12:35. 1885; *Caeomurus hyalinus* Kuntze, Rev. Gen. 3^3:450. 1898; *Telospora hyalina* Arth., Result. Sci. Congr. Bot. Vienne 346. 1906.

LITERATURE: SACCARDO, Syll. Fung. 7:581. 582. 1888; HARIOT, Revue Mycol. 14:21. 1892; SYDOW, Monog. Ured. 2:128. 1909.

This rust, first described upon *Sophora sericea* from Colorado, and made the type of the genus *Telospora*, has been found to possess uredinia. OLIVE, in his paper on intermingling of perennial sporophytic and gametophytic generations, etc. (Ann. Myc. 11:309. 1913), mentions that ARTHUR has called attention "to the fact that *Uromyces Sophorae* seems to possess a similar habit [that is, an intermingling of mycelia] to the perennial rusts under discussion." In any event, the presence of uredinia, in some cases at least, suffices to exclude this species from the short cycle forms.

UROMYCES PAVONIAE Arth., Bull. Torr. Bot. Club 31:1. 1914.—*Telospora Pavoniae* Arth., Result. Sci. Congr. Bot. Vienne 346. 1906.

LITERATURE: SACCARDO, Syll. Fung. 17:250. 1905; SYDOW, Monog. Ured. 1:59. 1909.

This rust, described upon *Malache scabra* B. Vogel (*Pavonia racemosa* L.) from Porto Rico and Jamaica, belongs with *Puccinia*

heterospora Berk. and Curt. An examination of the material shows that a very few 2-celled teliospores are present. *P. heterospora*, upon related Malvaceous plants, is characterized by the preponderance of 1-celled mesospores such as those upon *Pavonia*.

ARTHUR (Mycologia **9**:80. 1917) has given a brief discussion of the situation here. *Uromyces pictus* Thuem. upon *Abutilon* was also found to possess a few 2-celled teliospores and was placed with *Puccinia heterospora* by SYDOW (Monog. Ured. **2**:58 and 356. 1910).

UROMYCES MONTANA Arth., BOT. GAZ. **39**:386. 1905.—*Telospora montana* Arth., Result. Sci. Congr. Bot. Vienne 346. 1906.

The type collection of this species possessed also aecia, which were at the time considered to belong with *Uromyces Lupini* B. and C. Subsequent collections in Guatemala by KELLERMAN and HOLWAY, however, show the same association of aecia and telia; furthermore, these aeciospores are larger and thicker walled than the aeciospores of *U. Lupini*. The grouped arrangement of the telia and the thin walls of the teliospores and their germination at maturity indicate a short cycle form, but nevertheless it is considered probable that the aecia go with the telia. *U. elatus* Syd., also upon *Lupinus*, shows the same situation as regards association of aecia with telia resembling those of a short cycle form. I am indebted to Dr. MAINS of Purdue for work upon this species.

UROMYCES CUPANIAE Arth., Mem. Torr. Bot. Club **17**:131. 1918.—*Uredo cristata* Speg., Anal. Soc. Ci. Argent **17**:119. 1884.

This rust, although short-cycled, is excluded from this group, since, as noted by ARTHUR, it has marked affinities with other groups of rusts rather than with the group herein treated.

Conclusions

Eleven species of *Uromyces* possessing only telia and pycnia, or telia alone, are now considered to be present in North America. These are found especially in the higher and warmer portions of the continent, and occur upon 7 widely separated host families. While these rusts form a group agreeing as to life cycle and as to the 1-celled character of the teliospores, it is not considered that phylogenetic interrelationship is thereby shown, morphological evidence indicating rather that the relationship of a

species of these rusts is found in other rusts (of various life cycles and with 1 or 2-celled teliospores) upon the same or related hosts. Indeed, as indicated under *Uromyces heterodermus*, a group of hosts may bear a number of rusts of various life cycles, belonging to *Puccinia* and *Uromyces*, widely distributed geographically, yet all showing a certain unanimity of morphological characters, especially in the telial stage.

The writer wishes to express his keen appreciation to Dr. J. C. ARTHUR for suggesting this paper and for much help, and also to Professor JACKSON for many suggestions and constructive criticism. To the other workers in the laboratory at Purdue University he is likewise greatly indebted.

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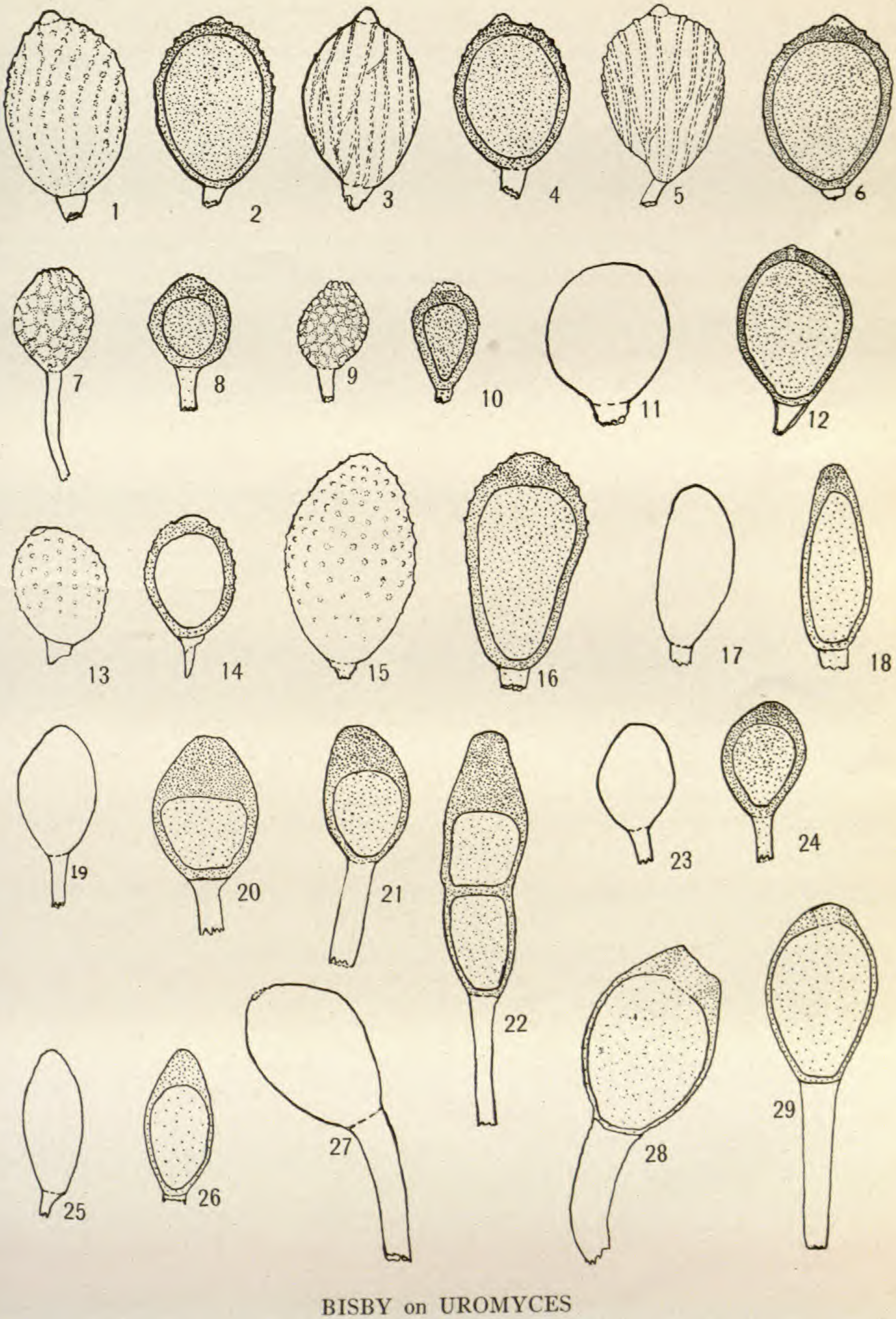
EXPLANATION OF PLATE X

All figures were drawn at the level of the stage with the aid of a camera lucida, with Leitz 1/12 oil immersion and ocular 4. They are here reduced one-third, so that the magnification is 667 diameters. Surface markings, where present, are indicated, and the stippling on the optical cross-section diagrams



BISBY on UROMYCES

represents to some degree the comparative darkness of color of the spore walls. In most cases the drawings of short cycle *Uromyces* were made from type material.

FIGS. 1, 2.—Surface and optical cross-section, respectively, of teliospores of *Uromyces heterodermus* Syd., from type material, on *Erythronium parviflorum*, Wasatch Mountains, Salt Lake County, Utah; A. O. Garrett, Fungi Utahensis *118*.

FIGS. 3, 4.—*Uromyces Erythronii* (DC.) Pass. on *Erythronium dens-canis*, Bohemia. Sydow, Ured. *1505;* species correlated with preceding.

FIGS. 5, 6.—*Uromyces Holwayi* Lagerh. on *Lilium columbianum,* Washington. Barth., N.A. Ured. *1387;* compare two preceding species.

FIGS. 7, 8.—*Uromyces bauhiniicola* Arth., from type material on *Bauhinia Pringlei*, Guadalajara, Mexico.

FIGS. 9, 10.—*Uromyces jamaicensis* Vesterg., from type material, on *Bauhinia* sp., Constant Spring, Jamaica.

FIGS. 11, 12.—*Uromyces abbreviatus* Arth., from type material, on *Psorlea Purshii*, Winnemucca, Nevada. Griffith, W.Am. Fungi *390*.

FIGS. 13, 14.—*Uromyces Tranzschelii* Sydow, from type material, on *Tithymalus* (*Euphorbia*) *montana*, Fossil Creek, Colorado. Ellis and Everhart, Fungi Columbiana *1069*.

FIGS. 15, 16.—*Uromyces nevadensis* Hark., from type material, on *Primula suffrutescens*, near Lake Tahoe, Nevada.

FIGS. 17, 18.—*Uromyces Myrsines* Diet. on *Ardisia compressa*, south of Cartago, Costa Rica.

FIGS. 19, 20.—*Uromyces Solidaginis* (Sommerf.) Niessl. on *Solidago serotina*, Helena, Montana. Ellis and Everhart, N.A. Fungi *2883*.

FIG. 21.—*Uromyces Solidaginis* on *Solidago virgaurea*, Sweden. Sydow, Ured. *2406*.

FIG. 22.—*Puccinia Asteris* Duby on *Aster adscedens*, Salt Lake County, Utah, illustrating correlation with *Uromyces Solidaginis*.

FIGS. 23, 24.—*Uromyces amoenus* Sydow, from type material, on *Anaphalis subalpina*, Paradise Valley, Mount Tacoma, Washington.

FIGS. 25, 26.—*Uromyces Rudbeckiae* Arth. and Holw., from type material, on *Rudbeckia laciniata*, Decorah, Iowa.

FIGS. 27, 29.—*Uromyces Bidentis* Lagerh. on *Bidens pilosa*, Ponce, Porto Rico, from type material of *Uromyces densus* Arth.

FIG. 28.—*Uromyces Bidentis* from material from type locality on *Bidens andicola*, Ecuador.

EFFECT OF SALTS UPON OXIDASE ACTIVITY OF APPLE BARK

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 263

D. H. Rose, Henry R. Kraybill, and R. C. Rose

(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 autooxidation 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.

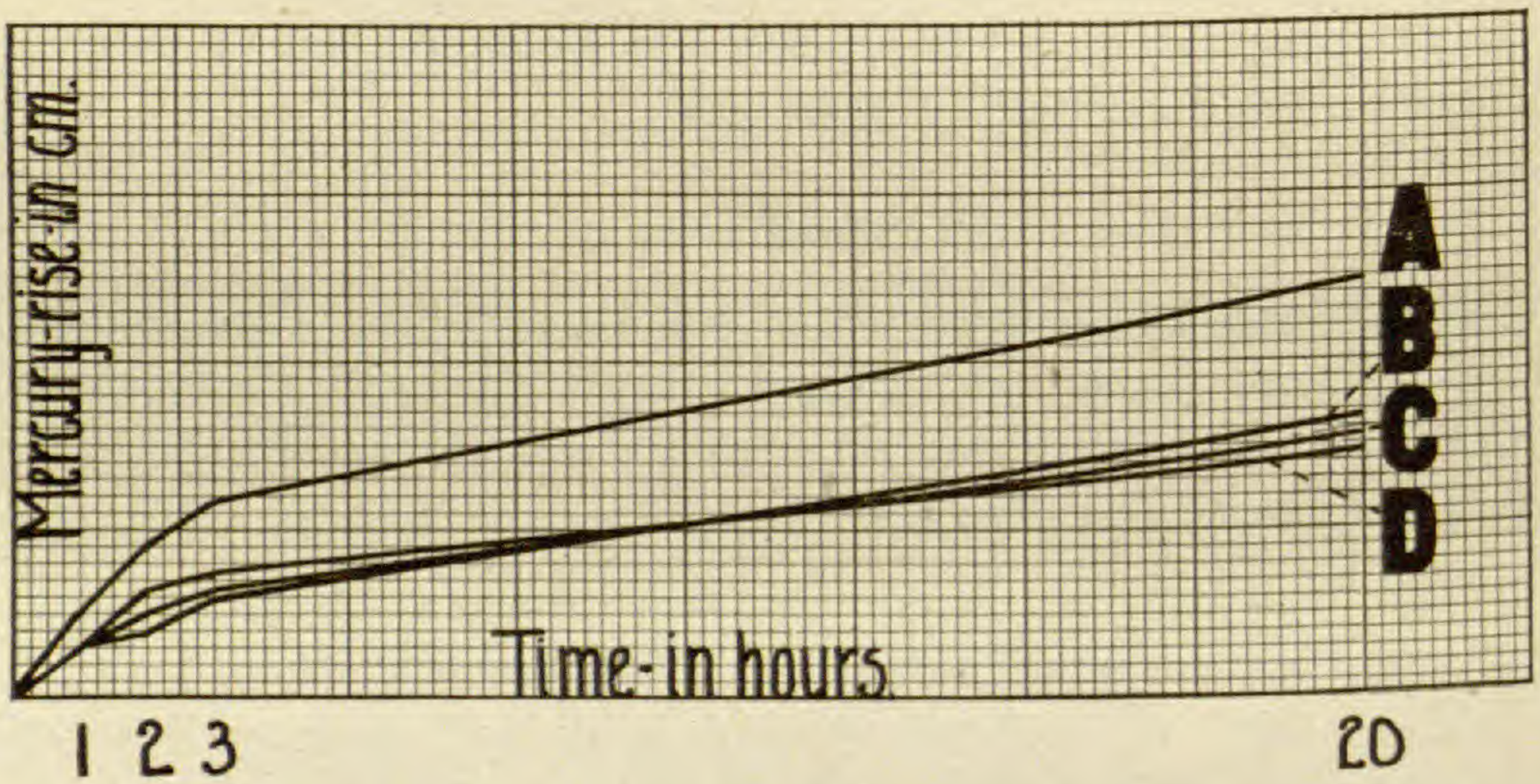


FIG. 1.—Effect of KCl, NaCl, and LiCl on oxidation of pyrogallol by powdered healthy apple bark: A, control (bark and pyrogallol); B, KCl+bark and pyrogallol; C, NaCl+bark and pyrogallol; D, LiCl+bark and pyrogallol.

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

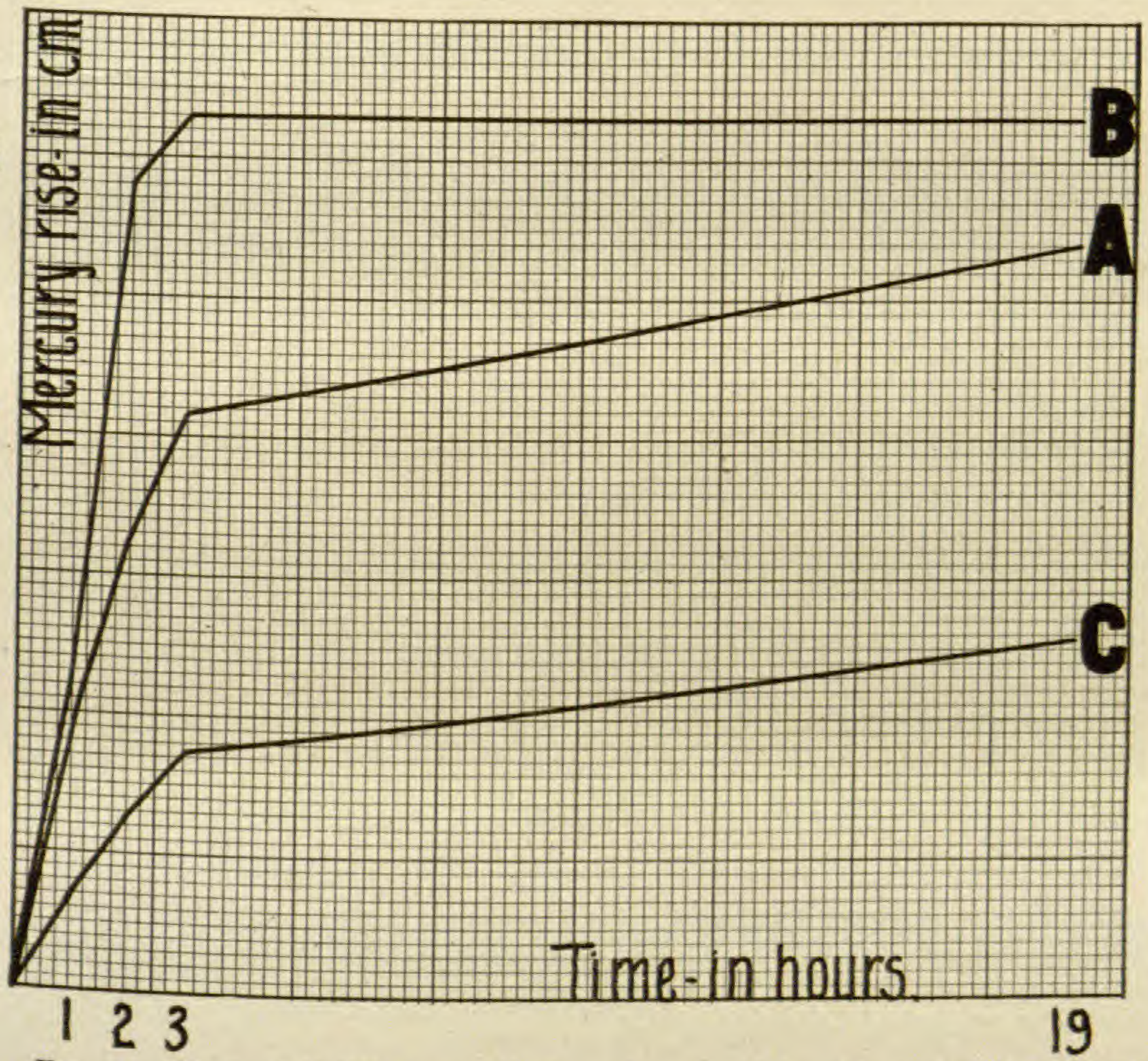


FIG. 2.—Effect of K_2CO_3 on oxidation of pyrogallol, with and without bark (healthy): A, K_2CO_3+bark and pyrogallol; B, K_2CO_3+pyrogallol; C, control (bark and pyrogallol).

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

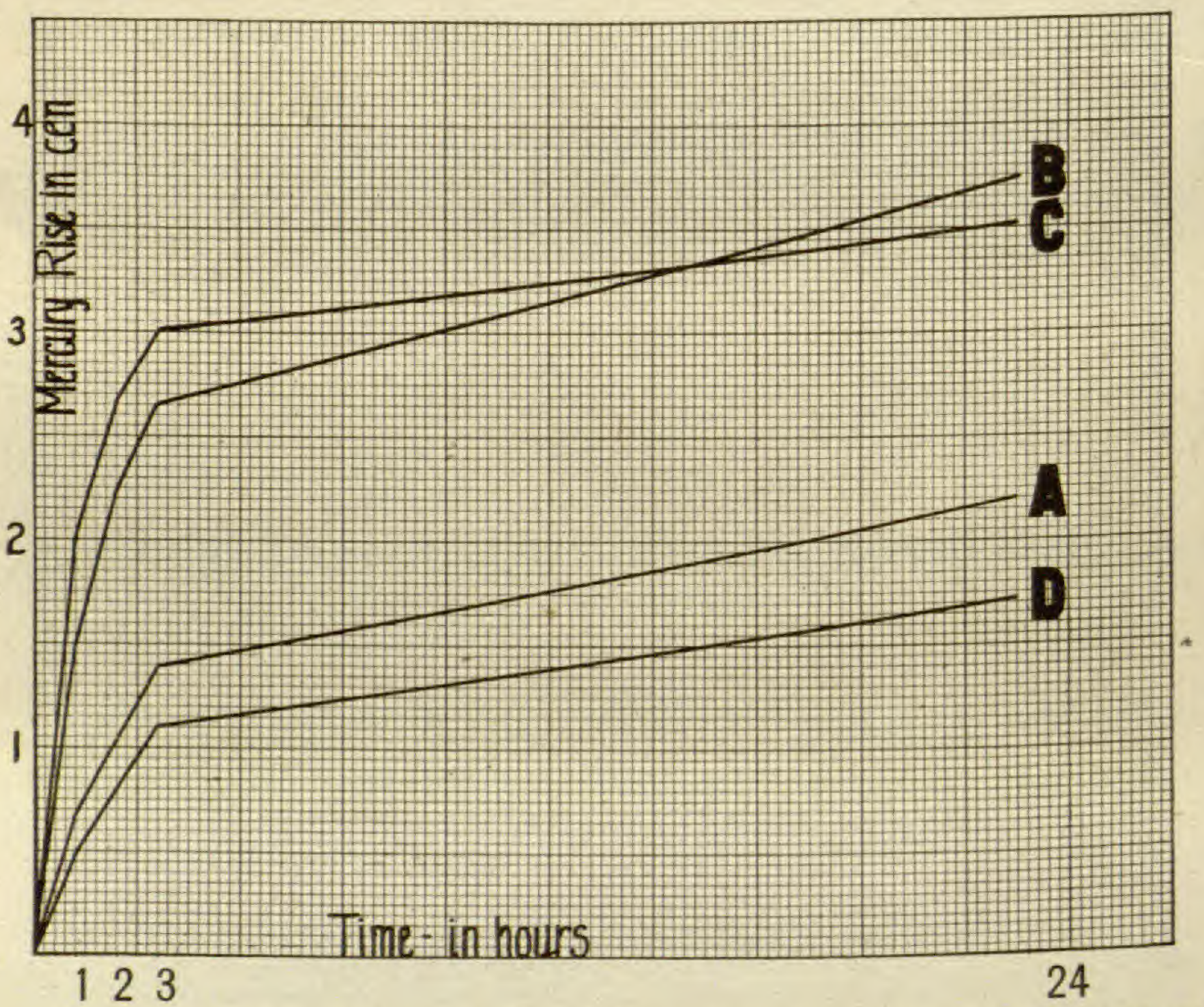


FIG. 3.—Effect of KCl and K_2CO_3 on oxidation of pyrogallol with and without bark (diseased): A, control (bark and pyrogallol); B, K_2CO_3+bark and pyrogallol; C, K_2CO_3+pyrogallol; D, KCl+bark and pyrogallol (KCl+pyrogallol gave no oxidation).

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

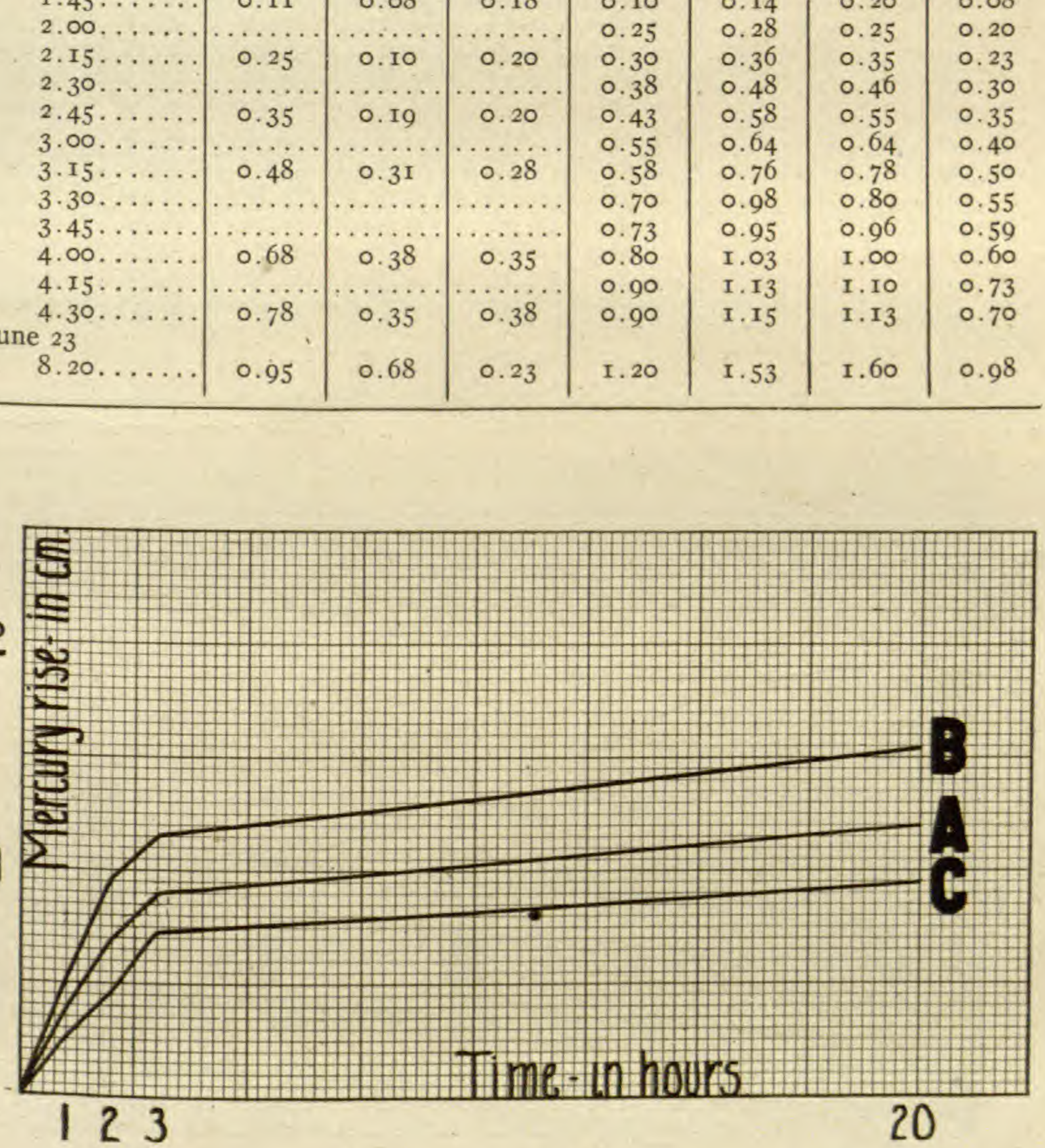


FIG. 4.—Effect of potassium tartrate on oxidation of pyrogallol with and without bark (healthy): A, control (bark and pyrogallol); B, potassium tartrate+bark and pyrogallol; C, potassium tartrate+pyrogallol.

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

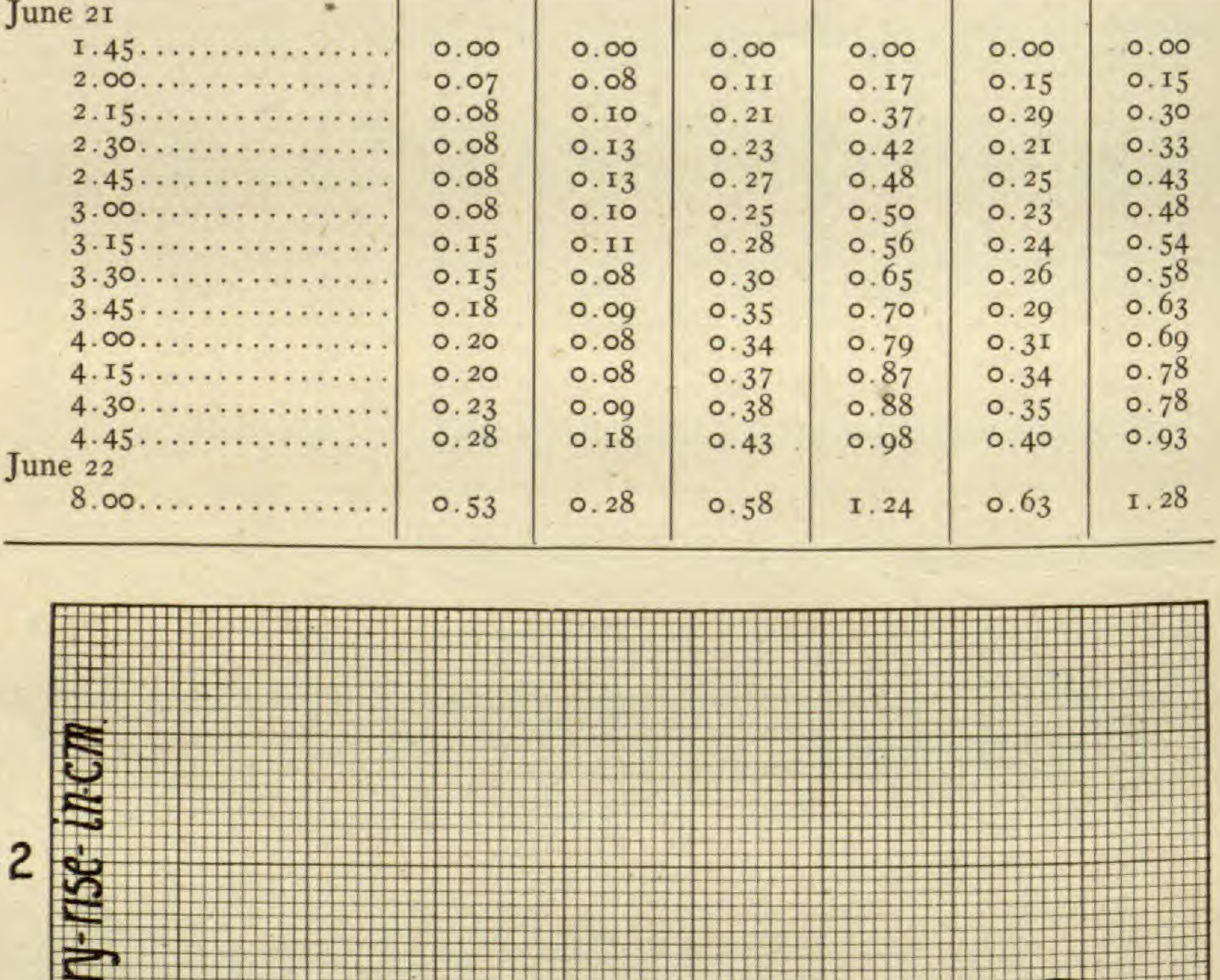


FIG. 5.—Effect of $MnCl_2$ and K_2SO_4 on the oxidation of pyrogallol on precipitated oxidase from both healthy and diseased bark: A (diseased), K_2SO_4+bark and pyrogallol; B (diseased), control (bark and pyrogallol); C (diseased), $MnCl_2$+bark and pyrogallol; D (healthy), K_2SO_4+bark and pyrogallol; 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 P_H 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.

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PIT-CLOSING MEMBRANE IN OPHIOGLOSSACEAE

GERTRUDE WRIGHT

(WITH PLATES XI, XII AND SIX FIGURES)

The members of the Ophioglossaceae, an isolated family of uncertain origin, are forms with a few large leaves, simple to decompound, and short, slow growing, underground stems, vertical, oblique, or horizontal in position, with crowded fleshy roots. The leaves, which are divided into sterile and fertile lobes, bear on the latter homosporous sporangia.

Of the three widely distributed genera, *Helminthostachys*, a monotypic genus, is the most restricted, occurring throughout tropical Asia to North Australia and New Caledonia. *Ophioglossum* is represented by about 30 species growing under various conditions of moisture and shade in the temperate and tropical zones of both the Eastern and Western hemispheres. *Botrychium*, with nearly as many species, is world wide in its distribution, but is confined chiefly to the temperate regions.

The forms considered in this paper are *Helminthostachys zeylanica*, *Ophioglossum vulgatum*, the only species of the genus native to Canada, and *Botrychium obliquum*, one of the 6 or 8 forms found in Ontario.

The rhizome of *Ophioglossum vulgatum* consists of a large, starch-filled cortex surrounding a siphonostele of endarch bundles of primary wood. This cylinder may be broken by leaf gaps, often so prolonged as to overlap, producing a circle of bundles. Fig. 1 shows several such bundles, one, beside an outgoing root, starting on its way through the cortex to the petiole. There is no endodermis in the mature plant, and the pith is directly continuous with the cortex through the large leaf gaps.

Helminthostachys, whose rhizome is horizontal and dorsiventral, presents a slightly different appearance in cross-section. Fig. 2 shows its broad woody cylinder solid on the lower side, broken

on the upper right by a relatively small leaf gap beside an outgoing leaf trace. The wood is entirely primary, with groups of parenchyma scattered throughout it. The mesarch structure of the bundles is not evident here, but may be demonstrated by means of longitudinal sections. The large-celled, winding endodermis is, unfortunately, too faintly stained to show clearly in the figure. According to FARMER and FREEMAN (4), there is in this form cork formation confined to the upper surface and originating at the bases of the cast-off leaves.

The most extraordinary member of the group in regard to its wood structure, however, is undoubtedly *Botrychium*. In this form there occurs a well developed cylinder of secondary wood, as well as a definite cork layer. The stem shown in transverse section (fig. 3) illustrates this. The woody cylinder surrounding a rather large starchy pith is solid with the exception of small leaf gaps, one of which appears in the lower part of the figure to the left of a horizontal root. The wood, which is composed of tracheids of irregular size, is traversed by numerous uniseriate medullary rays of slightly radially elongated parenchyma. The few and inconspicuous primary bundles are endarch. The pericycle consists of several rows of parenchyma, and is surrounded by an endodermis, frequently multiple. A rather large cortex, also utilized in the storage of starch, is bounded by cork which is visible in the upper right-hand corner of the figure.

The roots of the three genera show no secondary wood of any account. BOODLE (2) has described the addition of a few tracheids at the base of the old roots of *Ophioglossum vulgatum* and *Botrychium Lunaria*, but the later formed parts show only typically primary bundles, in the case of the former genus monarch in structure, and in the latter triarch or tetrarch (figs. 4, 5). The hexarch stele of the *Helminthostachys* root also shows only primary arrangement (fig. 6).

The character of the wood elements themselves in the three genera differs almost as much as their arrangement. Fig. 7 shows the elements in the metaxylem of the root of *O. vulgatum*, stained with Haidenhain's iron-haematoxylin and safranin. They do not differ from those of the stem, hence they represent the general

condition, pitting of the bordered scalariform type. With this stain the primary wall shows broad and black through the secondary, dividing the narrow red borders of adjacent pits. This is most apparent in the upper half of the tracheid to the left, where the scalariform openings are uniseriate, extending from side to side of the tracheid. In the lower half of the tracheid the primary wall has not been cut. The pit borders are more or less clear, also, about the middle of the tracheid to the right where the pits are small, oval, and biseriate. A combination of silver nitrate solution and ammonia, used with a counter stain of methylene blue, demarked these borders most clearly, but, unfortunately, did not lend itself to photography.

On the other hand, the metaxylem of *Helminthostachys* and the metaxylem and secondary wood of *Botrychium* exhibit a much greater differentiation. The tracheids, as seen in longitudinal section, are irregular and frequently nodular in appearance, with pitting distributed equally on their radial and tangential walls. The section illustrated in fig. 14 is from the rhizome of *B. obliquum*, cut tangentially and stained with haematoxylin and safranin. The tracheids are irregular in size and position, and interspersed with uniseriate medullary rays. The central tracheid shows the typical pitting of the secondary wall. The uniseriate and biseriate pits are large, round to oval in shape, with a centrally placed round pore. The small shaded area surrounding the pore is lignified.[1] In the tracheids to right and left is depicted a feature characteristic of both *Botrychium* and *Helminthostachys*, a tertiary wall of lignin. About the center of the tracheid to the left this layer appears as reticulately arranged bars lying over the pitted secondary wall. Above the center the plane of section is lower, exposing only the secondary wall; below the center it is through the lumen of the tracheid, and consequently the tertiary layer is seen in section. In the tracheid to the right, both the tertiary and secondary walls have been cut only in section. Fig. 15, also from *B. obliquum*, gives a sectional view of the pits with their overlaid scalariform. The pit cavities are approximately twice as

[1] In all the text figures lignification has been indicated by means of shading, and a different focus or an obscure feature by dotted lines.

long as broad, and rounded at the ends. The spools between, forming their borders, show a fairly thick, secondary, unlignified wall, ridged in most cases by one to two lignified (shaded) bars. The areas between the pits are small, and the primary wall which traverses them has, frequently, at the edges of the pits, thickenings

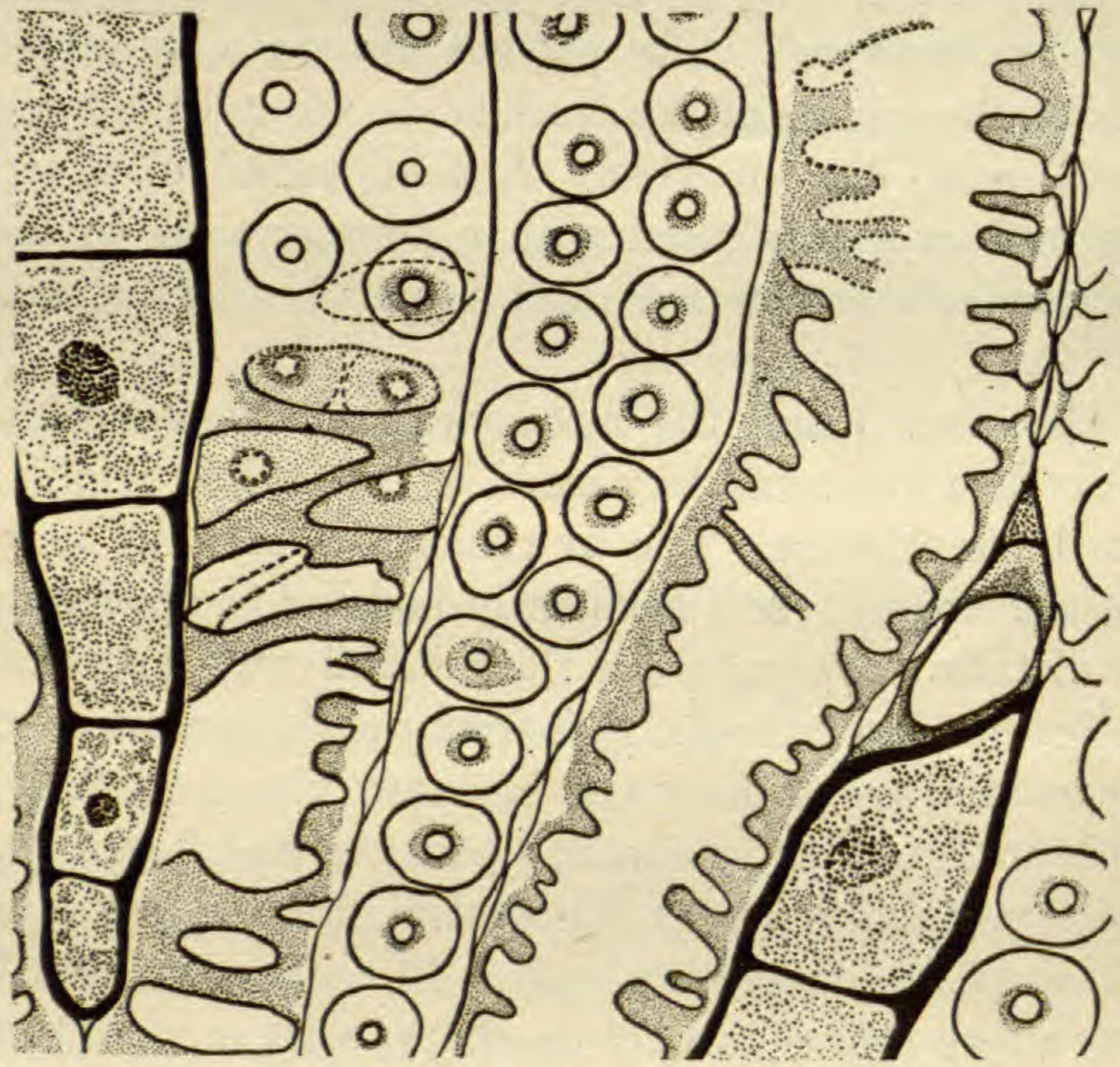

FIG. 14.—*Botrychium obliquum:* tangential section of the rhizome showing pitting; ×600.

similar to bars of Sanio. These are shown on the last four spools toward the top of the figure.

Fig. 10 shows the stem wood of *Helminthostachys* to be fairly similar to that of *Botrychium*, as seen in figs. 14 and 15. To the right of the center the walls of two adjacent tracheids have been cut in section. The left-hand wall is composed of only the secondary layer, which is characteristically thinner than in *Botrychium*, that to the right, of tertiary bars as well. The reticulate arrangement of these bars may be seen in the tracheid to the left of the center. The first-formed elements of the metaxylem of both *Helminthostachys* and *Botrychium* show less of a tertiary layer than the later

formed ones figured here. The scalariform bars in the former are fine and rather far apart, in the latter broader and joined in such a way as to produce the reticulate effect of fig. 10.

In both *Botrychium* and *Helminthostachys* the tracheids of the root wood, although slightly smaller and more regular than those of the stem, resemble these very closely. There is, perhaps, a greater amount of open scalariform tertiary thickening than in the stem and less of the broad, close formation. The petiole wood of both forms is also a likeness in miniature of that of the stem, particularly of the first-formed elements of the primary metaxylem of the latter. Frequently, however, the pit pores in *Helminthostachys* petiole are long and oblique rather than round.

The presence or absence of a pit-closing membrane in the Ophioglossaceae, as in all the vascular cryptogams, has been a matter of dispute. RUSSOW (7), in illustrating his article of 1872, expressed the prevailing view of the anatomists of his time with regard to the vascular cryptogams in general, when he showed no membrane in the pits of either the side or the end walls of *Botrychium*. It was in the following year that SANIO, working with *Pinus sylvestris*, demonstrated beyond a doubt the presence, in the mature condition in that form, not only of a membrane but also of a torus. From that time the pendulum of opinion began to swing in the opposite direction. In response to the stimulus of SANIO'S discovery, evidence has steadily accumulated that the membrane in the vascular cryptogams remains in the pits of the mature wood, not only in the side walls of the elements but, with few exceptions, in the end walls as well. In 1908, however, this view was challenged by GWYNNE-VAUGHAN (5). In returning to the idea of the earliest investigators, that the membrane disappears through resorption in the mature wood, the author distinguishes two types of ferns, represented by *Pteris* and *Osmunda* respectively. Ferns of the *Pteris* type, he claims, lose their limiting membrane only from the pit cavities, while those of the *Osmunda* type lose it also from between the walls of adjacent tracheids in the region between the pits. GWYNNE-VAUGHAN describes a further modification of this type which, however, need not be discussed here, as he classes the Ophioglossaceae with ferns of the *Pteris*

type. As far as the longitudinal walls are concerned, the opposite view, that of the persistence of the membrane in the pits, was upheld by HALFT (6) for "all the vascular cryptogams." He demonstrated by physical and microchemical means the presence of a limiting membrane in both the side and end walls of a large number of ferns. HALFT'S work was verified in the following year by Miss BANCROFT (1), then a research scholar in the University College of Nottingham. Judging from her very lucid paper and my own results with members of this group, I should think that HALFT had shown the "real" nature of the elements in the ferns. Comprehensive as is his work, however, his statement is more so, for no mention is made of a study of any member of the Ophioglossaceae. Miss BANCROFT, also, in corroborating his work, omits this family.

Fig. 16, a drawing from the rhizome of *Ophioglossum vulgatum*, shows the typical membrane in that form. In sections stained with silver nitrate and ammonia and counterstained with methylene blue, the open scalariform pits, with their narrow, pale greenish-blue borders, are traversed by a uniform pale brown membrane. Fig. 17 illustrates the same condition in the root. Here haematoxylin accentuates the broad primary wall within the spools, and stains only faintly the membrane in the pit. The latter, indeed, often appears to be somewhat lignified, taking to a certain extent the red stain of the lignified pit borders. The petiole as it leaves the rhizome exhibits a similar type of membrane.

It was with the greatest difficulty that the membrane in *Helminthostachys* was stained sufficiently for clear demonstration. After prolonged staining with the ordinary haematoxylin and safranin solutions, it remained so vague that its presence only, but not its form, could be ascertained. The latter was finally revealed by a stain consisting of malachite green, Martius' gelb, and acid fuchsin, originally used by Dr. PIANEZE for cancer tissue. The stain was recommended by R. E. VAUGHAN (Ann. Mo. Bot. Gard. May, 1914) as a differential stain for fungus and host cells.

Fig. 18 shows the condition in the rhizome. The lignified (shaded) areas appear bright green, bounding the red of the unlignified secondary walls, which in turn bound the more deeply

stained red primary wall. In the pits, the unlignified membrane, a pale red, assumes the form of a long spindle-shaped torus. Fig. 8 is from the adult metaxylem of the root, and shows the torus lying across the space where the walls of adjacent tracheids have been torn apart in sectioning. A slight thickening was found also in the first-formed elements of the metaxylem. The tracheids

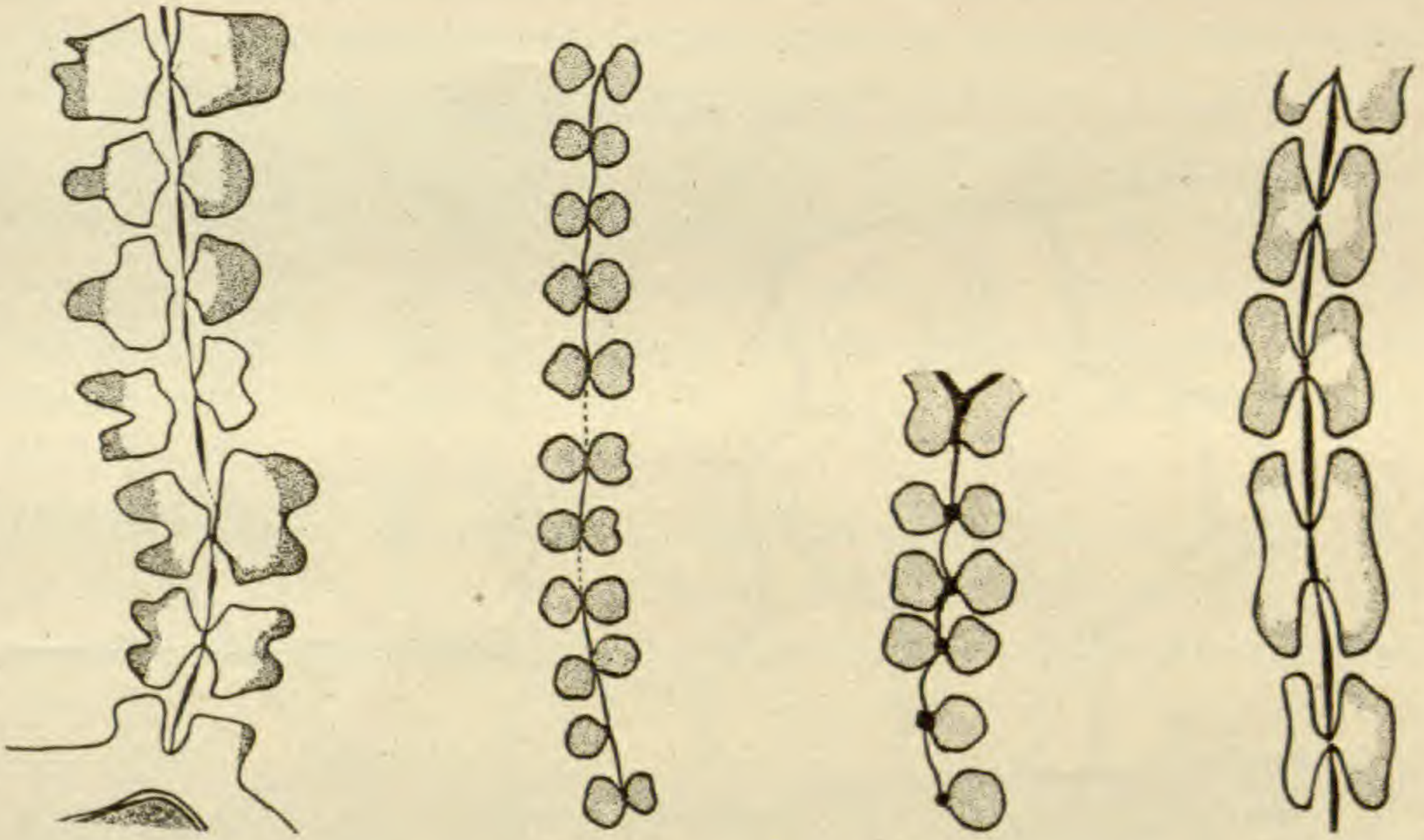

FIG. 15 FIG. 16 FIG. 17 FIG. 18

FIGS. 15–18.—Fig. 15, *Botrychium obliquum:* radial section of the rhizome showing pitting and torus; ×1200; fig. 16, *Ophioglossum vulgatum:* rhizome showing pit membrane; ×900; fig. 17, *Ophioglossum vulgatum:* root in cortex showing membrane; ×900; fig. 18, *Helminthostachys zeylanica:* longitudinal section of rhizome showing torus; ×900.

of the petiole in longitudinal section, however, show a fine uniform membrane with only occasionally a slight thinning toward the edges of the pit.

In *Botrychium* two types of torus occur. The most common type is that seen in figs. 9 and 15, a long, slender, and rather variable spindle. This is found in the mature wood of the stem, the root, and the leaf trace in the cortex. In the last region the membrane varies from a spindle to a uniform line, as seen in transverse section in fig. 11. The arrow in a tracheid to the right of the center points to a fairly thick membrane of the uniform type.

Fig. 12 shows a number of the spindle-shaped ones at a higher magnification. The pit pores have been outlined for greater clearness. The second type of torus occurs in the immature wood of the stem and occasionally in the root. Fig. 19 shows a transverse section from the cambial region of a young rhizome of *B. obliquum*. The tracheids are only slightly lignified, some still showing the contents. Here the torus is a short oval structure as

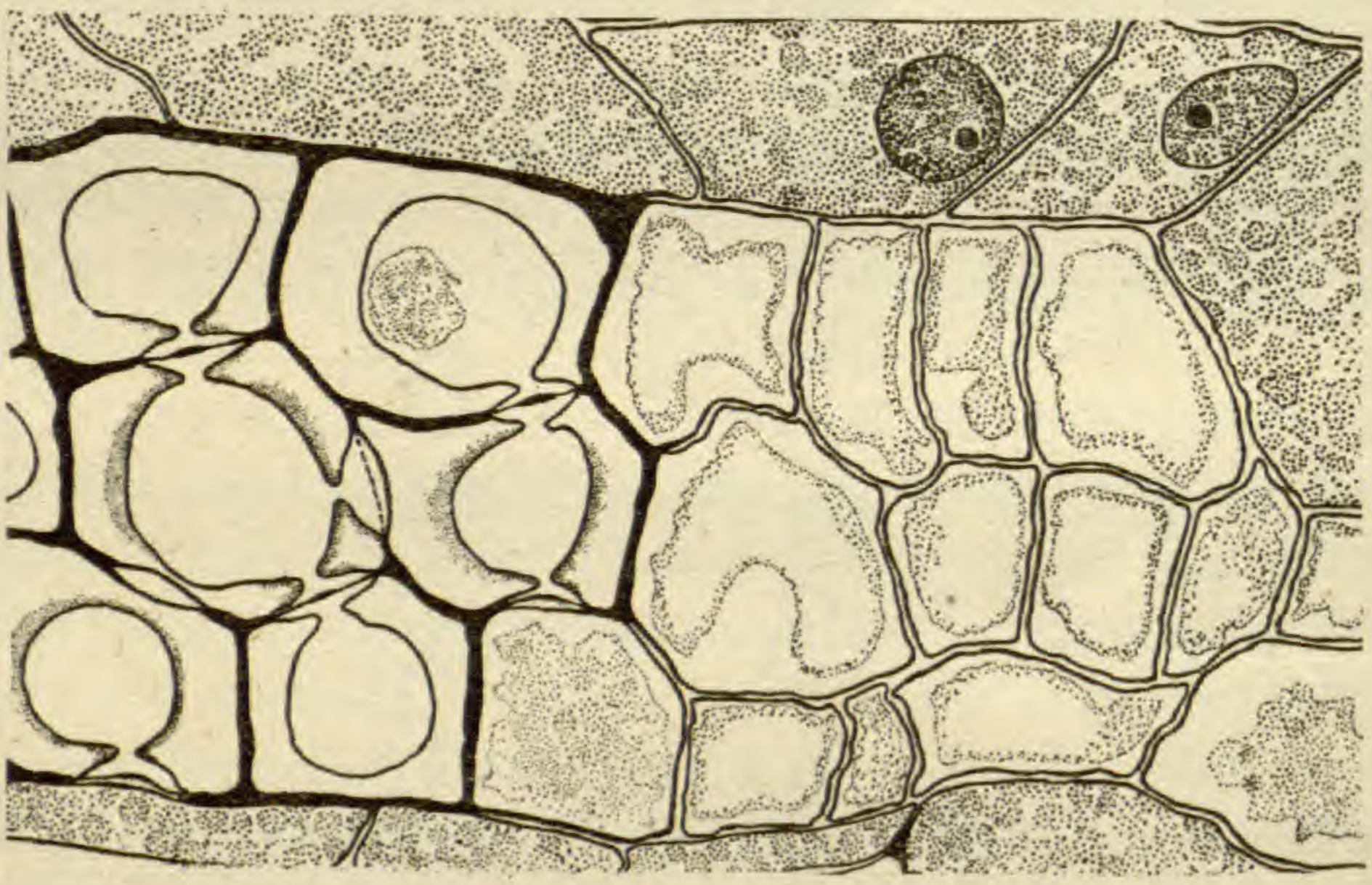

Fig. 19.—*Botrychium obliquum:* transverse section of young rhizome, at cambium showing torus and double membrane; ×600.

long as, or slightly longer than, the pore of the pit, and connected to its edges by a fine membrane.

This section (fig. 19) also illustrates a feature which I have observed in other forms, that is, the double nature of the membrane. In the pit of the tracheid at the left-hand lower corner the membrane is of a double character. The tracheid lies against a parenchyma cell of the ray, and only the half of the membrane next to the wood cell has been thickened, while that lying next to the ray cell remains uniform. The same double nature and plano-convex thickening of the membrane are shown in the third cell to the right. Here a tracheid, as yet unlignified and filled with contents, is adjacent to one which is more advanced in development, and the thickening occurs only on the side of the latter.

A peculiar condition is occasionally met with in the stem. The tracheids are more or less discolored when cut, and stain in a peculiar manner. With Pianeze's stain the membrane becomes yellow. It is usually uniform in thickness, but swollen, occasionally almost entirely filling the pit (fig. 13).

In the petiole of *Botrychium*, as in that of *Helminthostachys*, a uniform membrane prevails. With the exception of the petiole, therefore, and peculiar unnatural spots in the stem, the typical pit membrane in *Botrychium* has a torus.

Thus the only torus I have found among the cryptogams occurs in forms whose pits are broad-bordered and circular or oval in shape. STRASBURGER (8) makes the statement that a torus occurs in *Pteris aquilina*, but he neither enlarges on the statement nor illustrates it. DEBARY (3) describes and pictures for *Pteris* an almost imperceptible one-sided swelling of the membrane, lying to one side of the pit and acting, he states, as a lid to the pit pore. I have searched in vain for such a torus. Frequently the membrane may have a "kink" toward the pit pore simulating the appearance of a torus, but both its edges follow the curve to an equal extent, thus precluding the possibility of a thickening at that point. In *Pteris* the membrane in the pits between tracheid and tracheid invariably remains uniform in thickness. As has been shown in *Botrychium*, a plano-convex torus such as DEBARY describes may occur in the pits of a tracheid where it touches a ray cell. In *Pteris*, however, the membrane even in this region remains consistently uniform. *Equisetum*, *Psilotum*, and *Isoetes*, forms with narrow-bordered pits of the scalariform type, and a number of ferns (including *Ophioglossum*), with the same type of pitting, all show a definitely uniform membrane. In *Helminthostachys* and *Botrychium*, whose pits are circular, broad-bordered, and round-pored, there is developed a definite torus. Although this suggests a possible relation of the torus to the form of the pit, the question of its relationship, whether structural, ecological, or phylogenetic, is one on which it is hoped more light may be thrown after a study of the nature and occurrence of the torus in the other groups of the plant kingdom. It is interesting to note, however, that the form of the torus in *Botrychium*

and *Helminthostachys*, whose pitting is strikingly similar to that of the seed plants, resembles closely the type I have found in the lower gymnosperms, in *Ginkgo* and the araucarians, forms which are to be described later.

To Professor R. B. THOMSON, under whose direction this work has been carried on, is due my grateful acknowledgment of his invaluable assistance and advice. I am indebted also to both Professor THOMSON and Professor J. H. FAULL for material, some of which was obtained originally through the kindness of the Director of the Royal Botanic Gardens, Kew.

UNIVERSITY OF TORONTO

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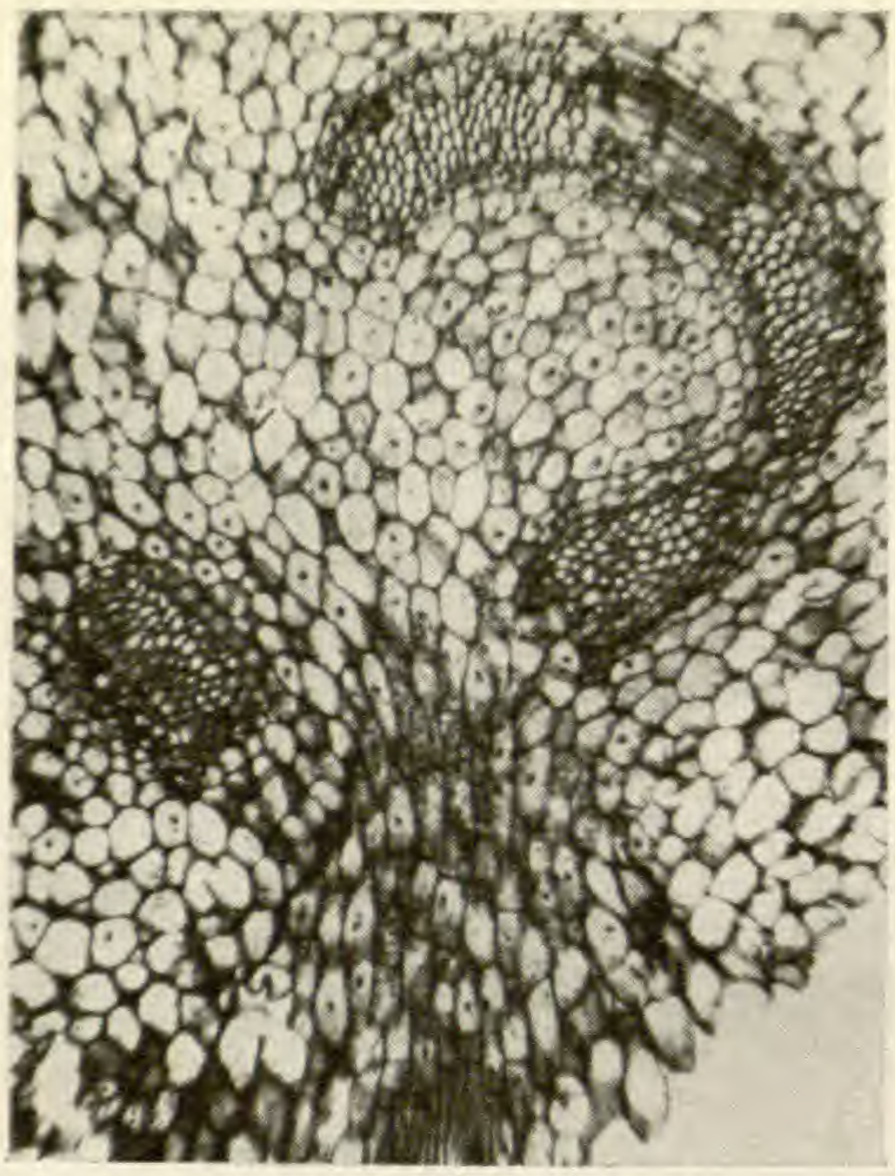
EXPLANATION OF PLATES XI, XII

FIG. 1—*Ophioglossum vulgatum:* transverse section of rhizome; ×50.
FIG. 2.—*Helminthostachys zeylanica:* transverse section of rhizome; ×35.
FIG. 3.—*Botrychium virginianum:* transverse section of rhizome; ×40.
FIG. 4.—*Ophioglossum vulgatum:* root, transverse section; ×210.
FIG. 5.—*Botrychium virginianum:* root, transverse section; ×120.
FIG. 6.—*Helminthostachys zeylanica:* root, transverse section; ×140.
FIG. 7.—*Ophioglossum vulgatum:* metaxylem of root showing pitting; ×875.



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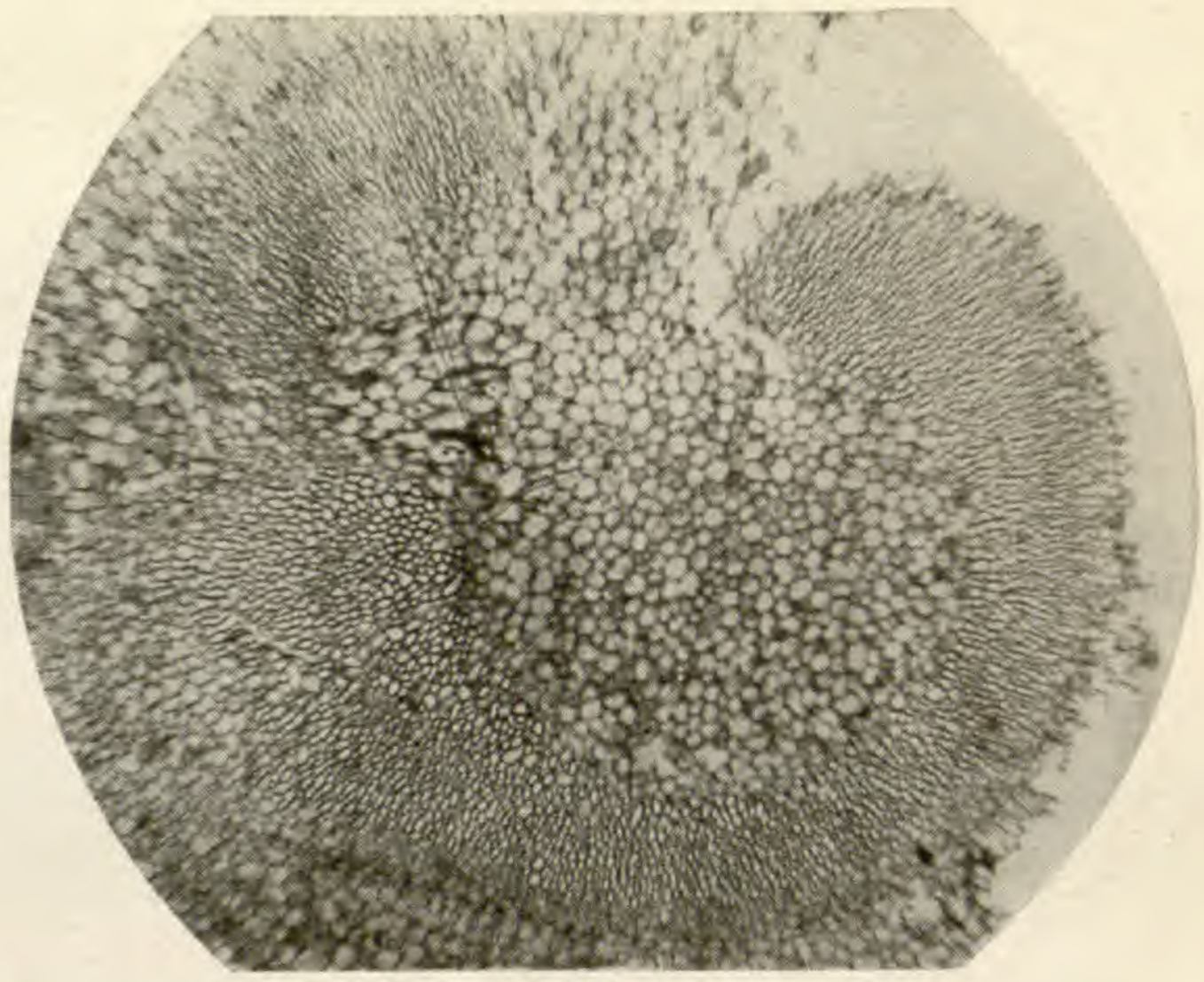

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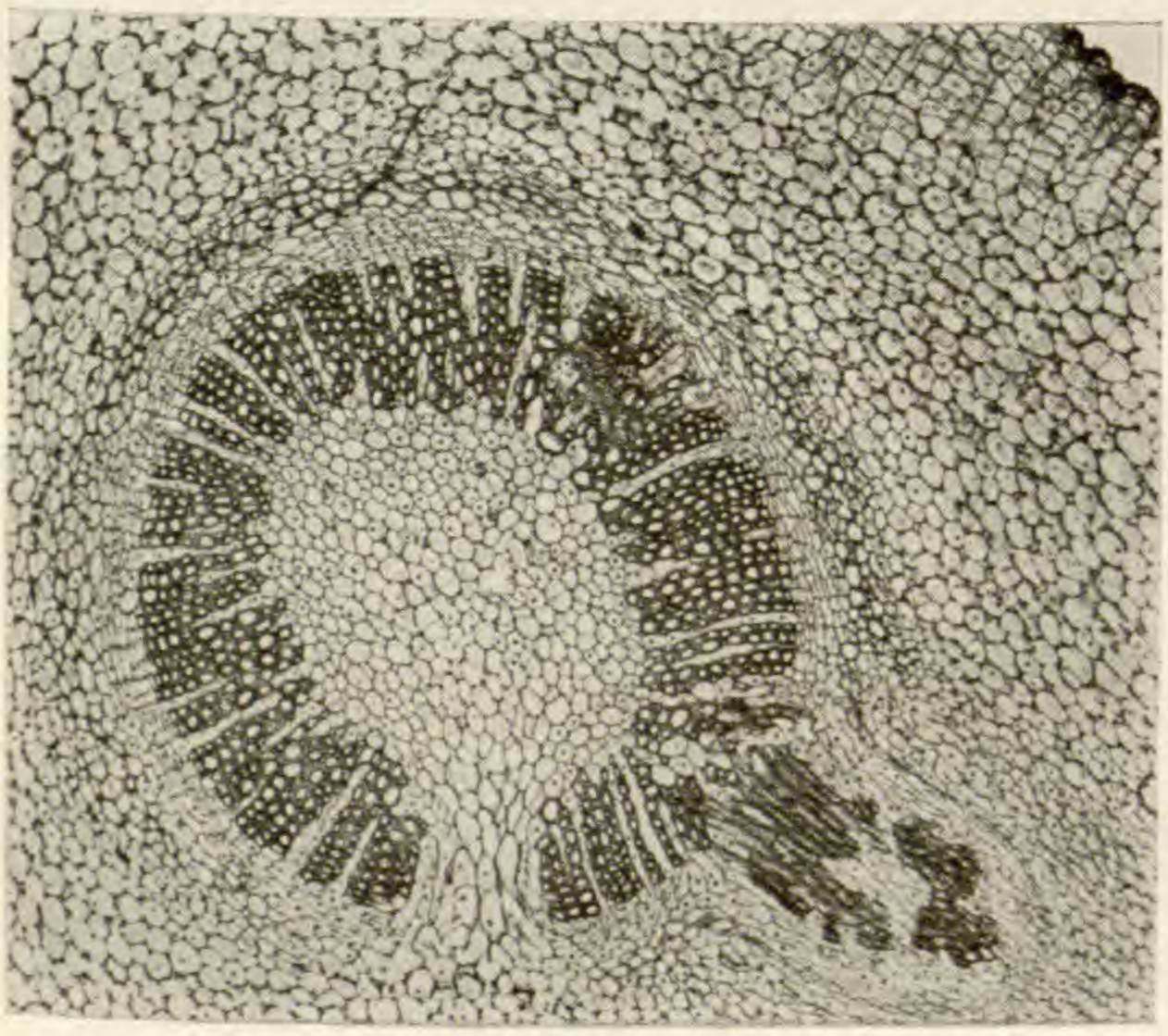

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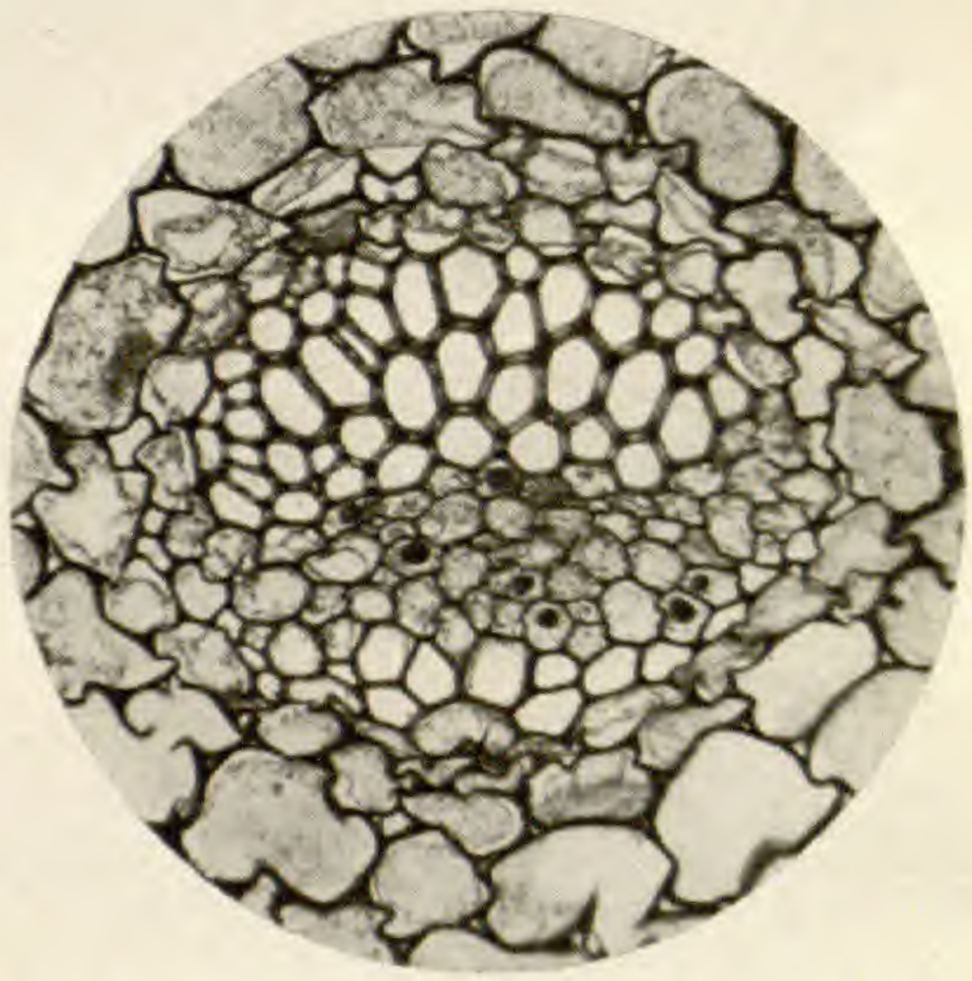

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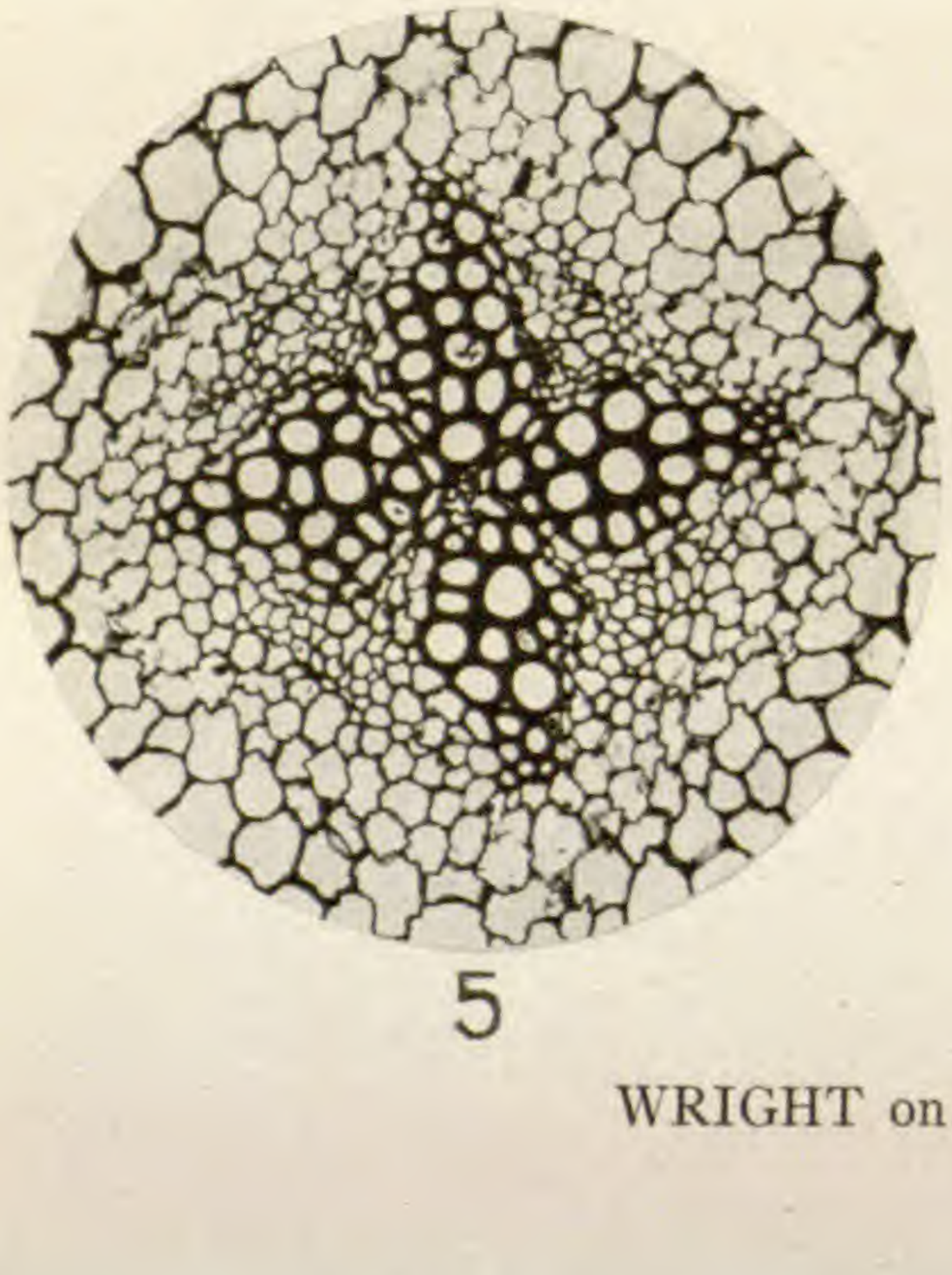

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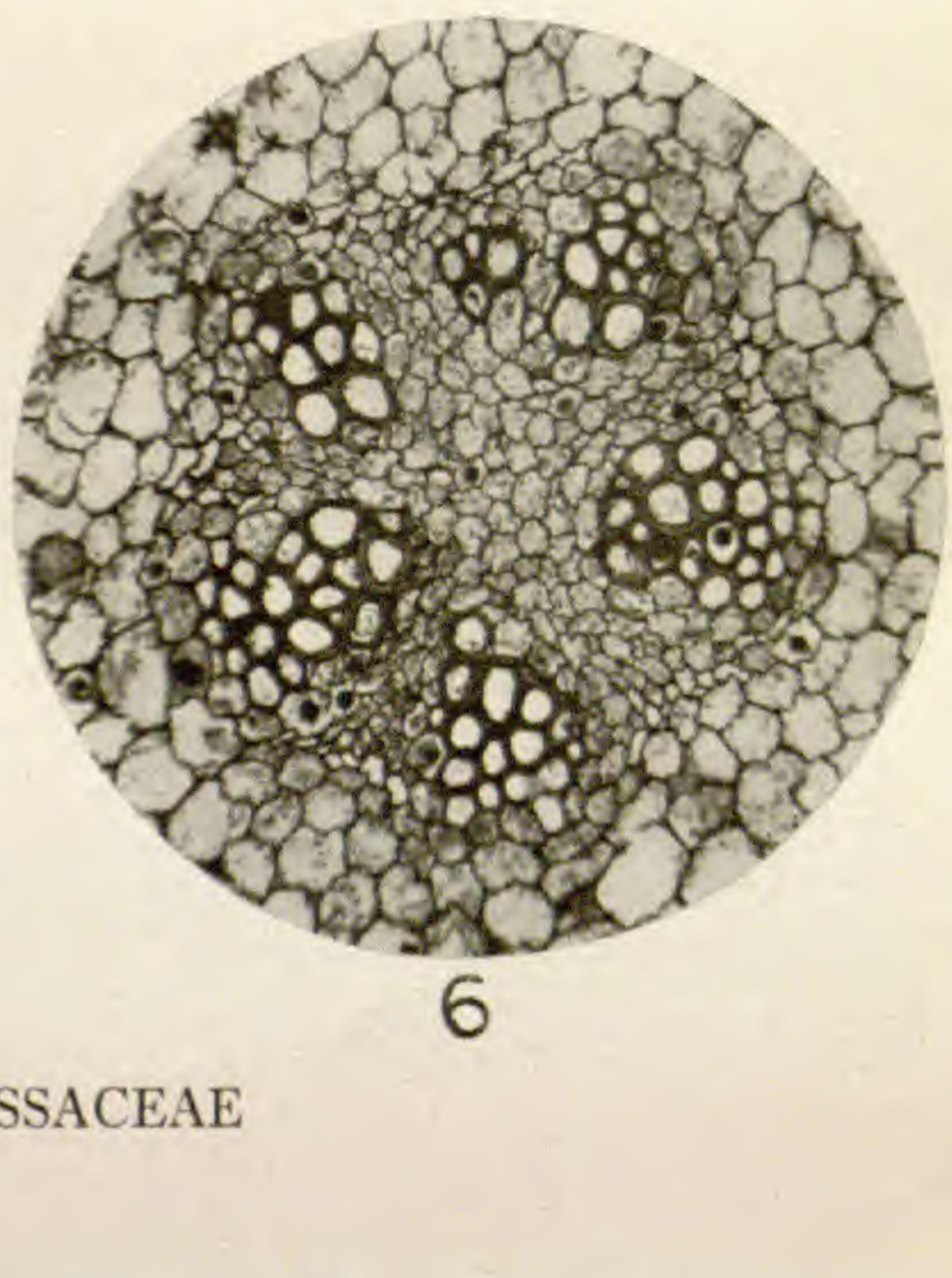

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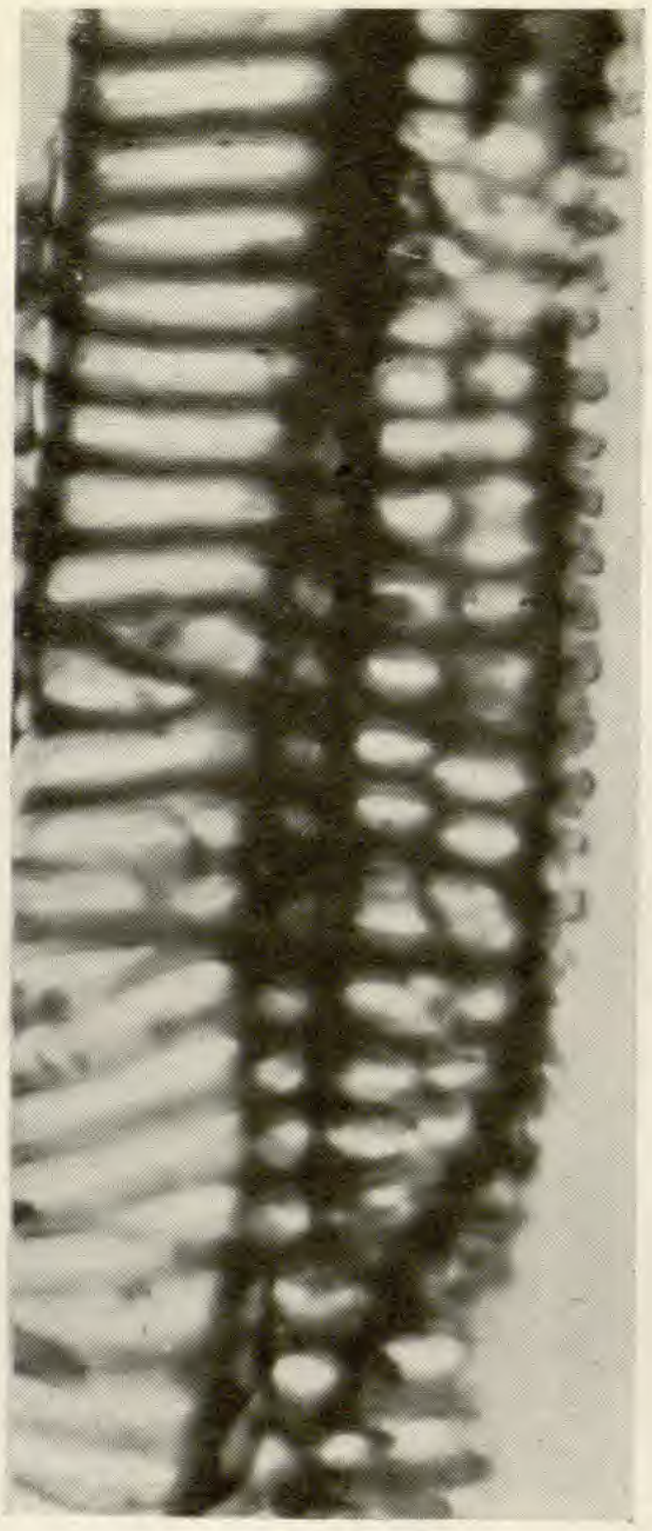
WRIGHT on OPHIOGLOSSACEAE



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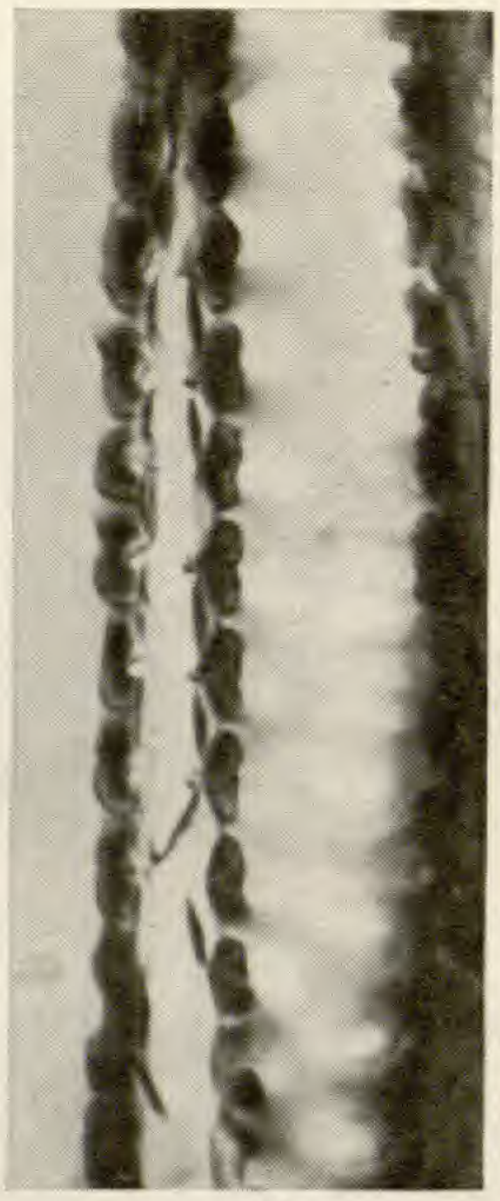

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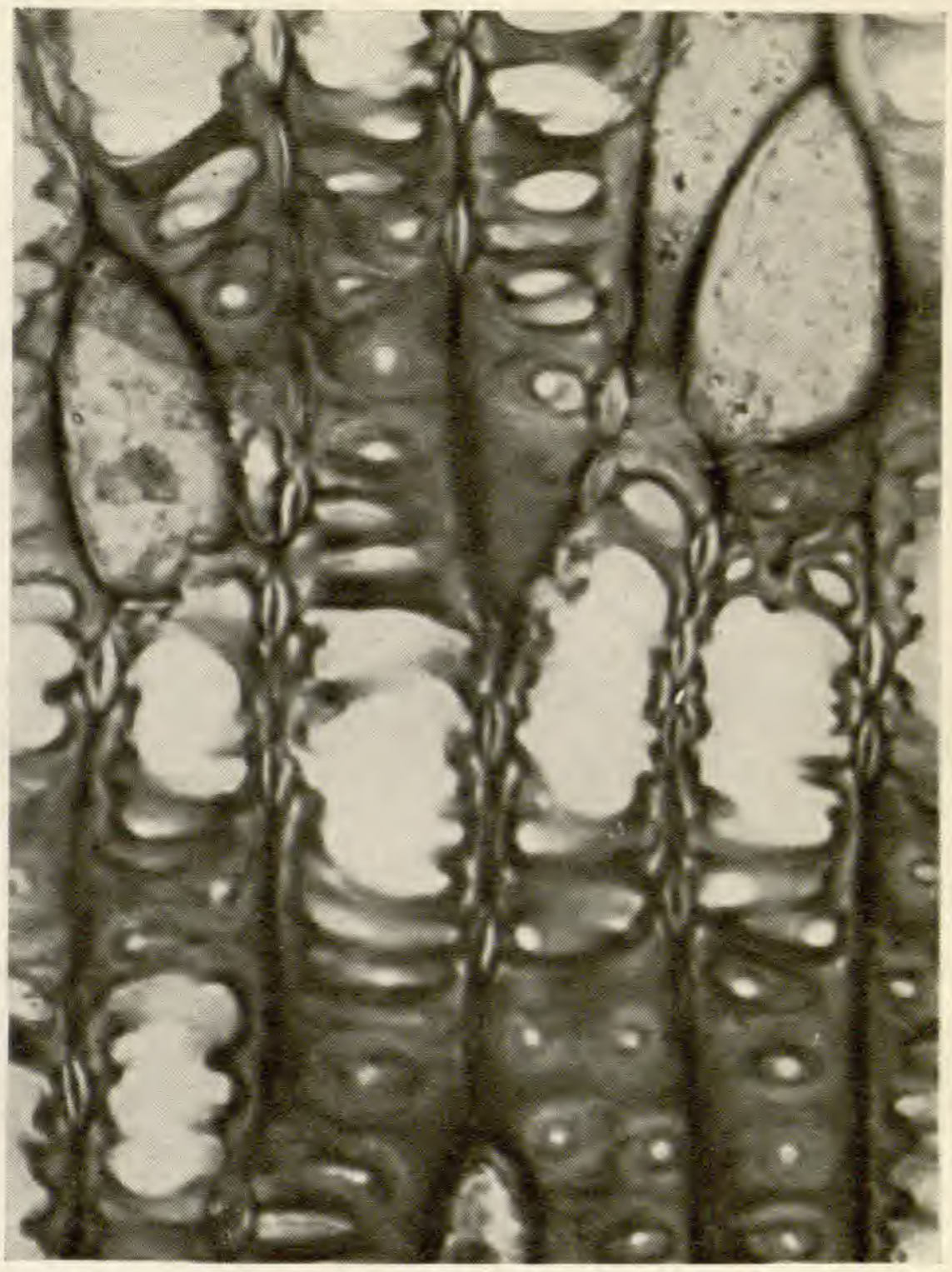

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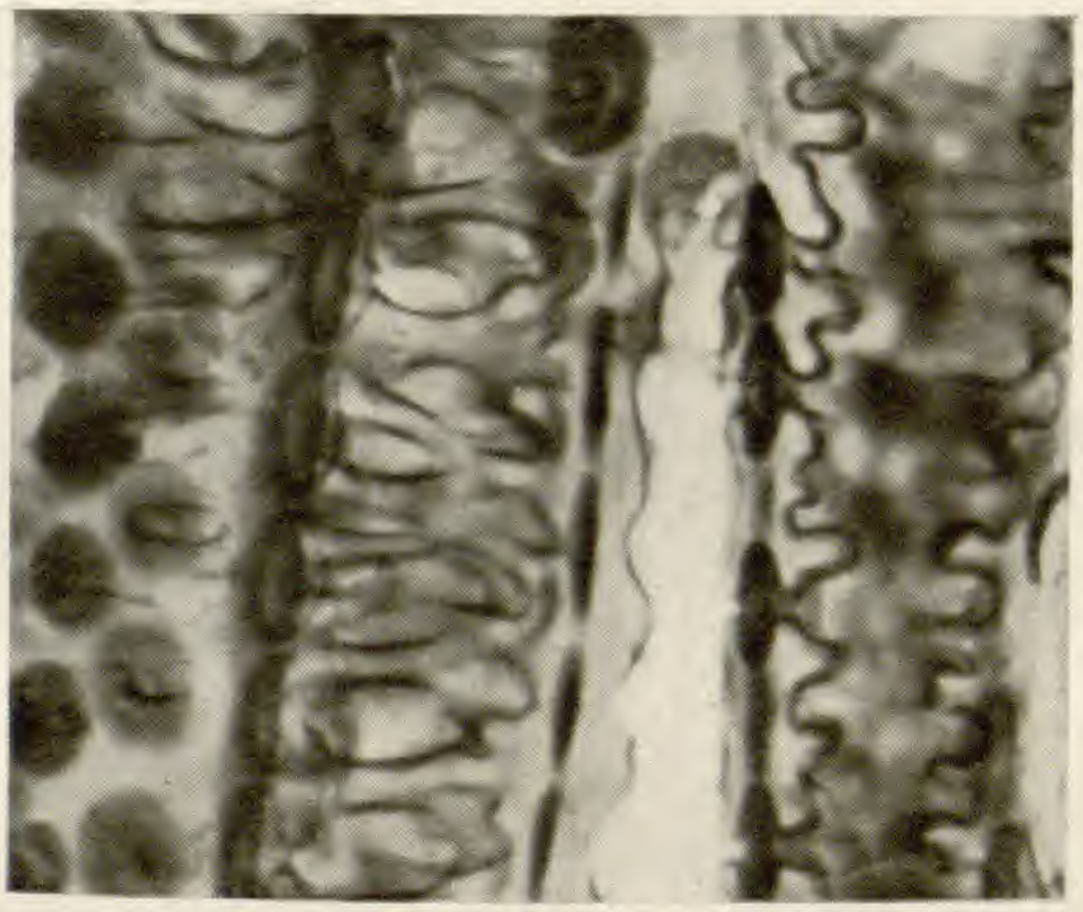

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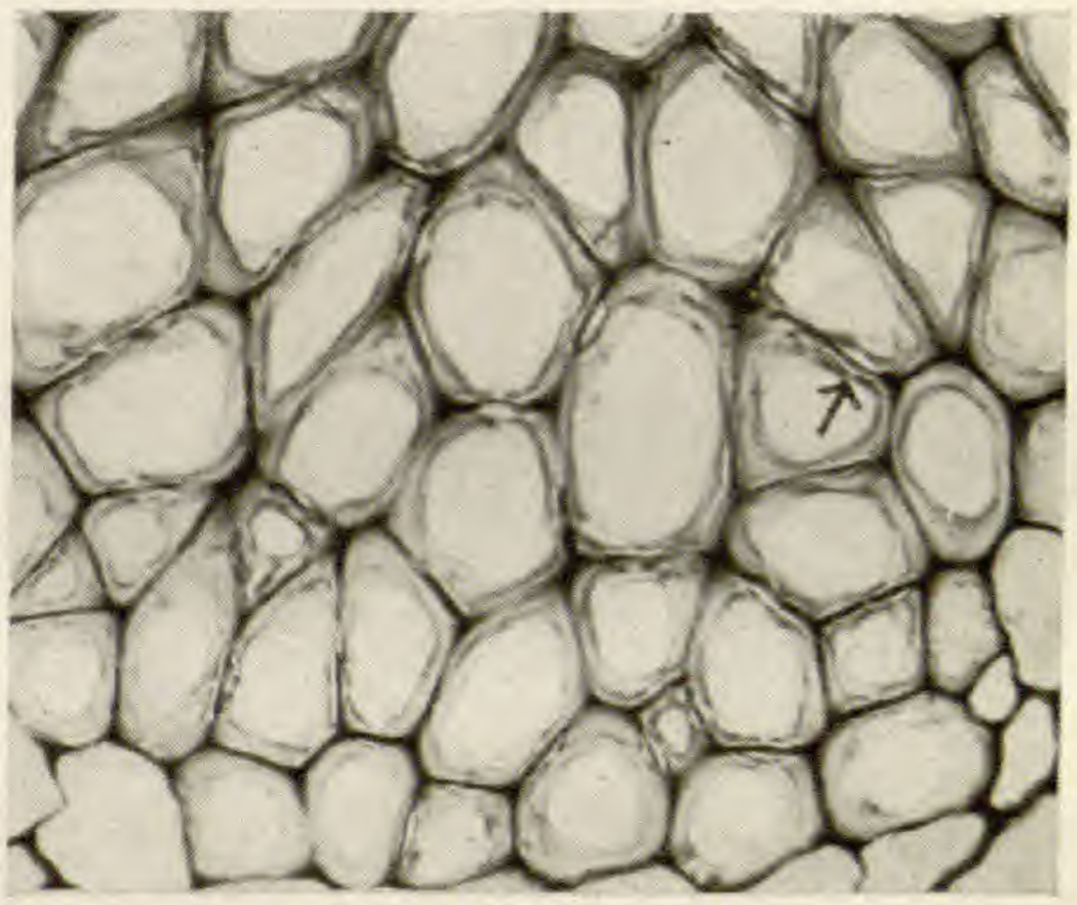

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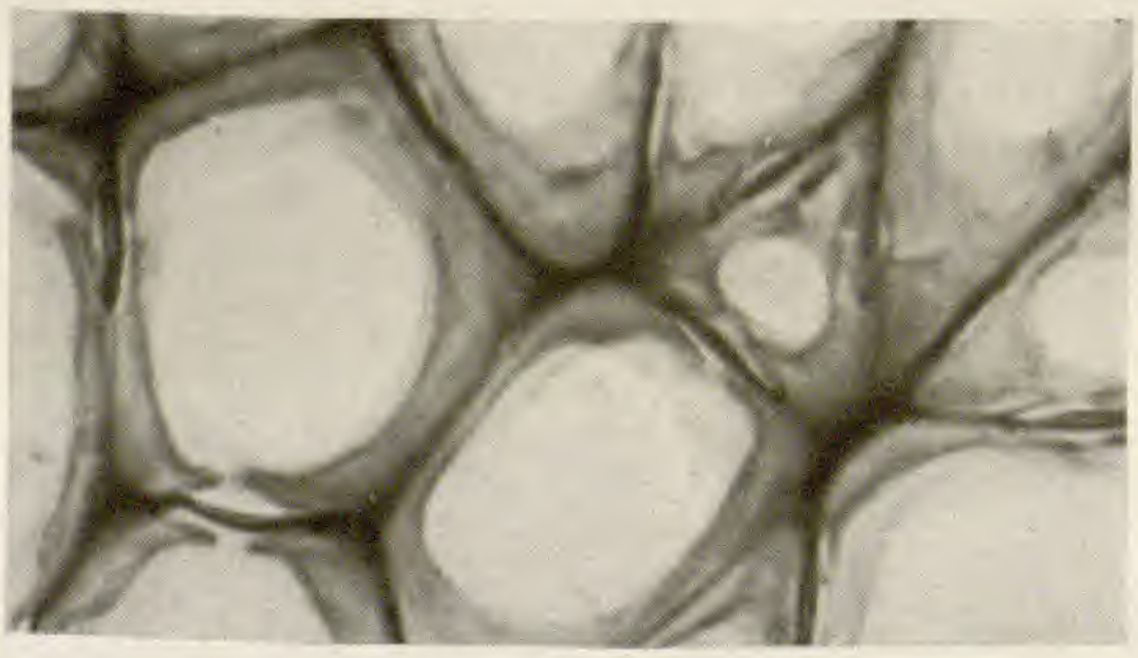

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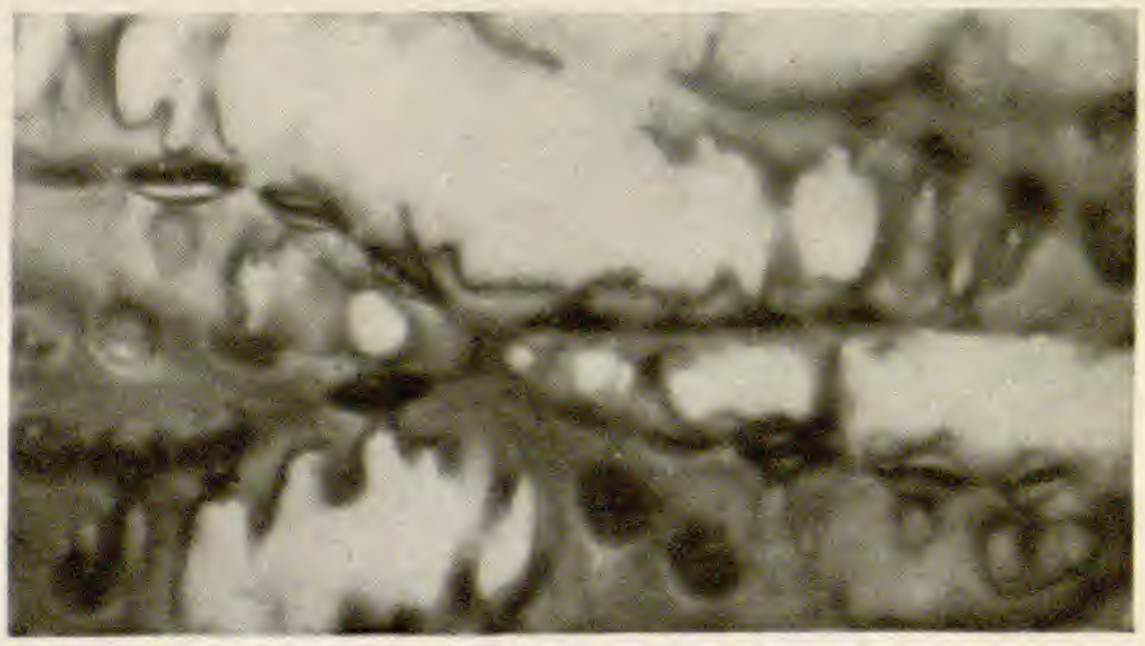

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FIG. 8.—*Helminthostachys zeylanica:* metaxylem of root showing torus; ×900.

FIG. 9.—*Botrychium obliquum:* rhizome, tangential section showing torus; ×735.

FIG. 10.—*Helminthostachys zeylanica:* rhizome, longitudinal section; ×825.

FIG. 11.—*Botrychium obliquum:* transverse section of leaf trace in cortex; ×425.

FIG. 12.—*Botrychium obliquum:* part of fig. 11 more highly magnified; ×875.

FIG. 13.—*Botrychium obliquum:* tangential section of rhizome showing thickened membrane; ×525.

DOTHIDIACEOUS AND OTHER PORTO RICAN FUNGI

F. L. STEVENS

(WITH PLATES XIII, XIV AND THREE FIGURES)

The following fungi were collected by the author in Porto Rico, and specimens are deposited in the herbarium of the University of Illinois, and of the New York Botanical Garden. The limitations accepted for the Dothidiaceous genera are those of THEISSEN and SYDOW,[1] which seem to be well founded and wholly tenable.

Dothideales

DOTHIDEACEAE

AUERSWALDIA CECROPIAE P. Henn. (figs. 4, 5).

On *Cecropia peltata:* El Alto de le Bandera, 9043; Mayaguez, 3931; Maricao, 8965; Rio Arecibo, 7798; Florida Adentro, 7756, 2475; Jayuya, 361; Añasco, 3581; Utuado, 6064.

This fungus, as the number of collections shows, is abundant in Porto Rico. From descriptions it seems to be the one just named. It is very variable in habit, especially with age, and there is some doubt as to its generic position. In young specimens there is no stroma, and the fungus appears Sphaeriaceous. In older specimens the stroma is well developed, and the fungus is clearly Dothidiaceous. No colored spores were seen, and the fungus to all appearances is really a *Phyllachorella.* Type material of *A. Cecropiae* P. Henn. and *Physalospora Cecropiae* Rehm are needed before a satisfactory decision can be made.

Uleodothis Pteridis, sp. nov. (figs. 6, 7).—Spots tan-colored, dead, 3–5 mm. across. Stromata black, rugose with perithecia, 1–2 mm. across, conspicuous above, less so below, slightly raised above the leaf surface, orginating sub-epidermally but eventually occupying the whole mesophyll, the upper surface rough and raised, without clypeus, and remaining covered by fragments of the epidermis. Hyphae of the stroma of general parallel arrangement. Loculi many, about 100 μ in diameter, globular. Asci

[1] Ann. Mycol. 13:149. 1915.

numerous, 65×14 μ, cylindrical, 4-spored. Paraphyses few, inconspicuous, fine, filamentous. Spores hyaline, 2-celled, oblong, 17–20×4–5 μ.

On *Pteridium caudatum*, Maricao, 4814 (type), 167.

This fungus agrees somewhat closely with *Dothidella pteridophila* Speg., but differs essentially in that it has paraphyses, and the asci are 4-spored. It differs from *Uleodothis*, as described, in having 4-spored asci, but it does not seem wise to found a new genus merely on this character.

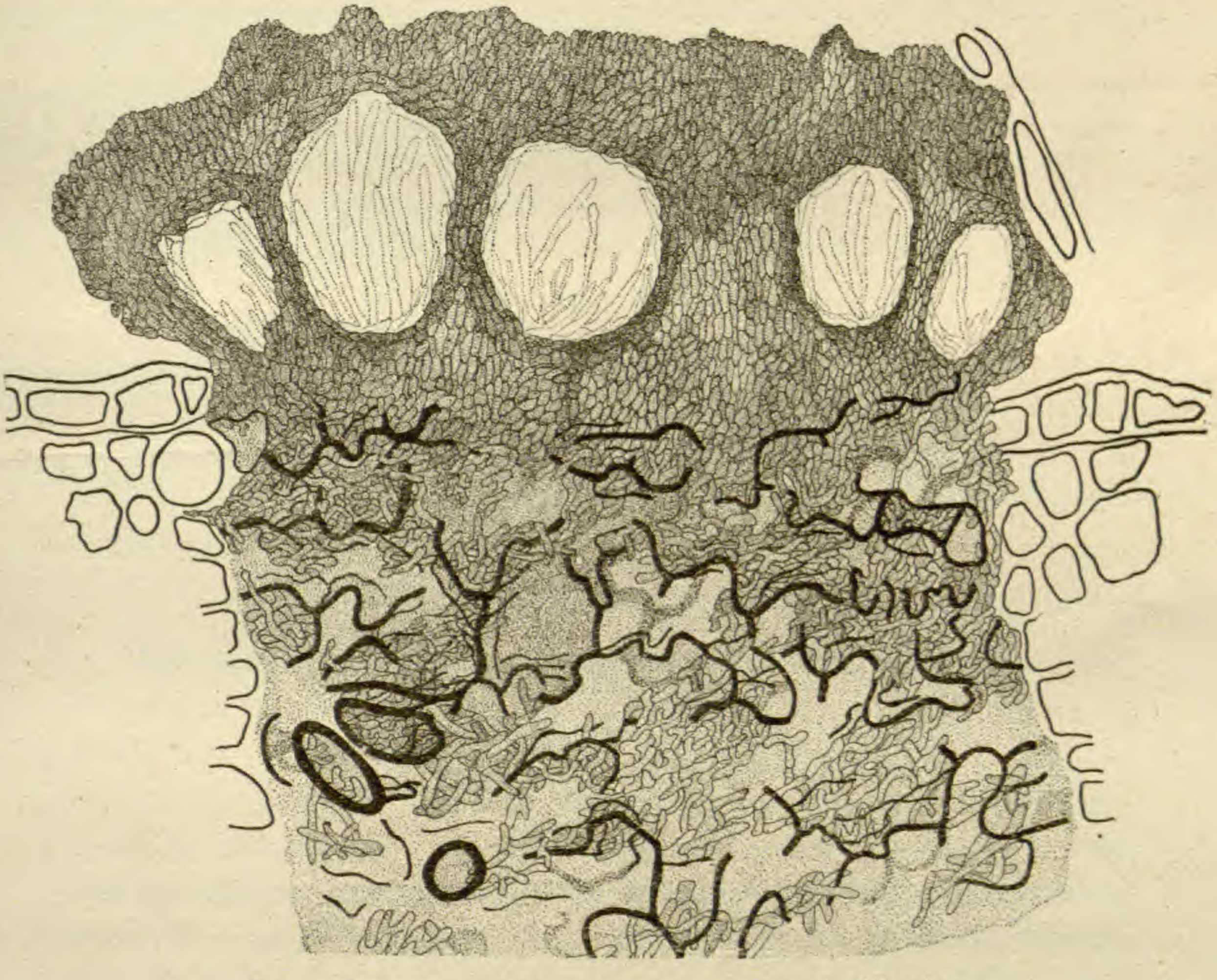


FIG. 1.—Structure of stroma and arrangement of locules

Dothidella portoricensis, sp. nov. (figs. 8, 9; text fig. 1).—Spots linear, 0.5–1×3–4 mm., amphigenous, definite. Stromata linear, entirely occupying the spots, raised above the leaf surface about 70 μ. Perithecial cavities in about 5 rows, nearly globular, about 70 μ in diameter. Paraphyses none. Asci numerous, cylindrical, 54×10 μ, 8-spored. Spores hyaline to dilute smoky, 1-septate, 17×3.5 μ.

On *Gleichenia*, Las Marias, 3551, x.62 (type).

The stromata differ essentially in shape from those of *D. pteridophila* Speg. (fig. 1).

Dothidella flava, sp. nov. (text figs. 2, 3).—Stromata pale to yellow, circular when young, linear when old; when mature, 1600 μ long by 270 μ wide, subepidermal, later erumpent, rising to considerable height above the leaf surface. Perithecial locules

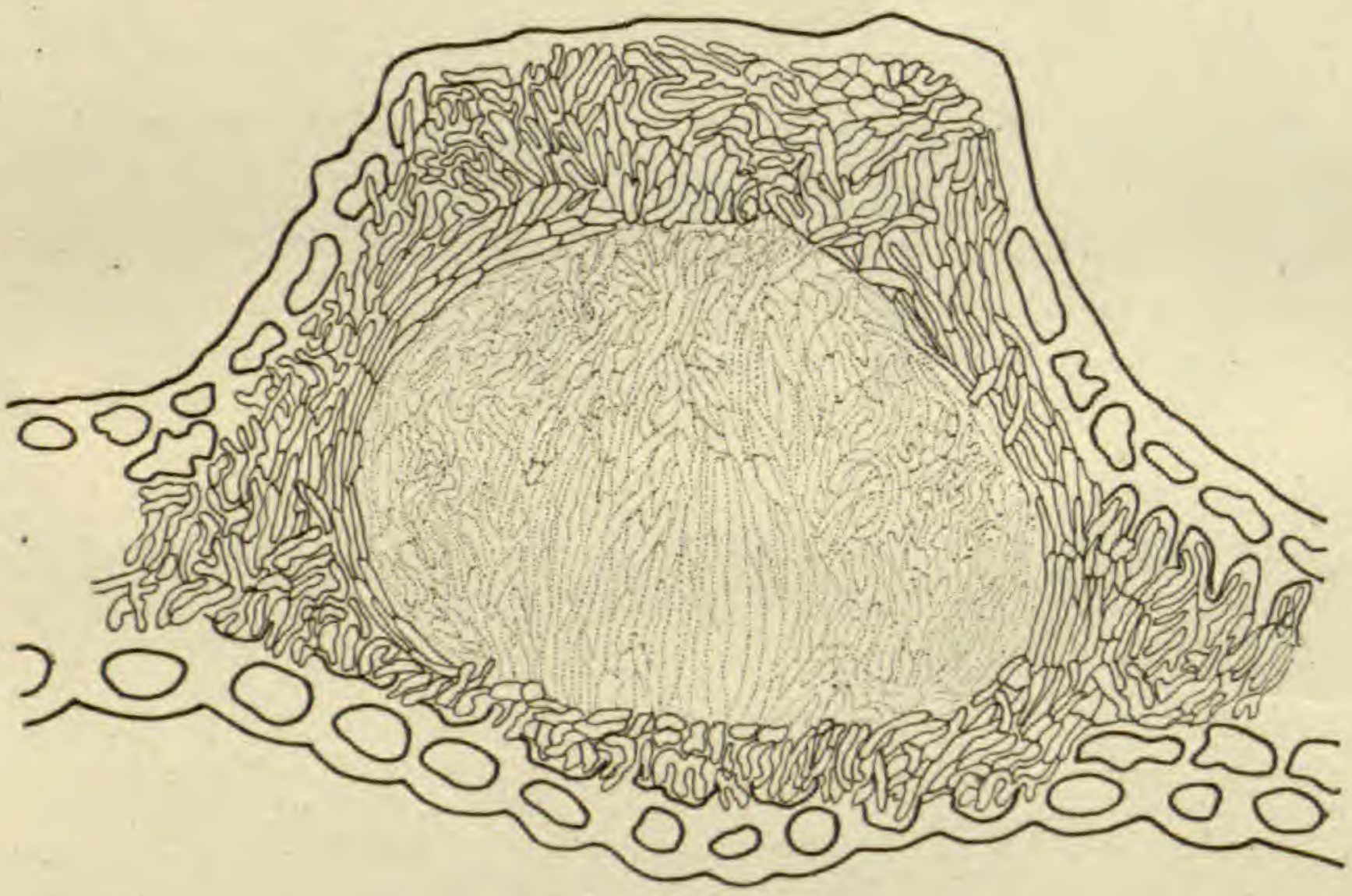


FIG. 2

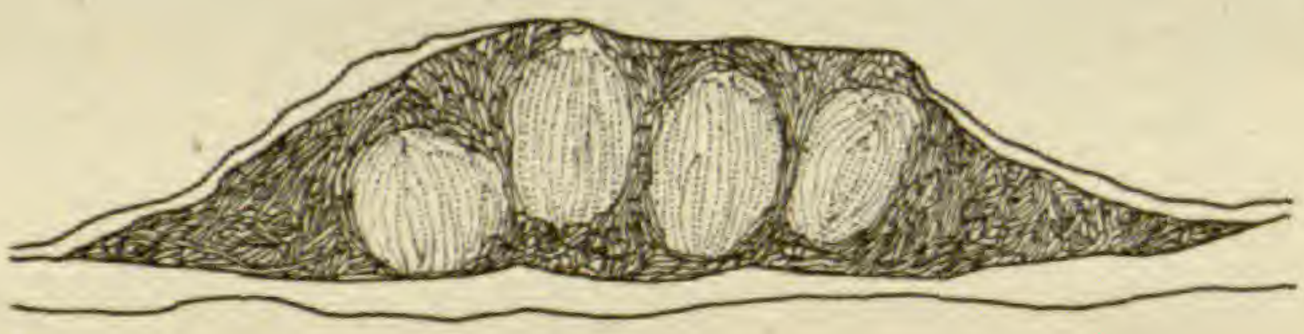


FIG. 3

FIGS. 2, 3.—Cross and long sections showing arrangement and shape of perithecia and pycnidia.

globular, 60–70 μ in diameter, arranged in one or two rows in the stromata. Asci linear, 8-spored, 34–51×6 μ. Spores hyaline, 2-celled, oblong, 3.5×14 μ. Conidia filamentous, 40×1.5 μ, hyaline, borne in the same stromata with the perithecia and preceding them, and either free in an acervulus or in pycnidial locules in the stroma.

On *Lithachne pauciflora:* Trujillo Alto, 9394, 7654; Mayaguez, 1062, 7432; Florida Adentro, 7665 (type), 7650.

This fungus is particularly interesting. To the naked eye it is like a rust. Superficial microscopic examination shows circular conidial sori which on casual observation might pass as a *Cylindrosporium*. Intermingled with the circular sori are many linear sori and stromata, all bearing the *Septoria*-like conidia in great numbers. Microtome sections show that all development is subepidermal. The first locule is conidiiferous, and is so thin-walled that the wall might easily be overlooked. The sorus elongates, ruptures the epidermis, and in section takes on the appearance shown in text fig. 3. At about this period perithecial locules appear with asci and spores. Their walls are indistinguishable from the surrounding stroma, and the whole structure is truly Dothideaceous. The stromata are frequently overgrown by a *Helminthosporium*. Following THEISSEN and SYDOW, it belongs to the Dothideaceae, falling in the genus *Dothidella*. To many it may appear more reasonable to put it in the Hypocreales on account of its color; but it appears to me to show much closer relationship with the Dothideaceae, notwithstanding its pale color.

PHYLLACHORACEAE

TRABUTIA RANDIAE (Rehm.) Th. and Syd.

On *Randia aculeata*, Cabo Rojo, 6455.

This fungus is clearly a *Trabutia* with strictly subcuticular stroma, and as it agrees well with the published description of *T. Randiae*, it is in all probability that species.

SCIRRHIINEAE

Catacauma Ocoteae, sp. nov.—Spots irregularly circular, 0.5–1 cm. or more in diameter, visible from above or below on dead, tan-colored tissue, border indefinite. Stromata circular, numerous, scattered throughout the spot, plane above, strongly rounded below, 1–1.5 mm. in diameter, between the lower epidermis and the mesophyll. Clypeus hypophyllous, rarely epiphyllous, extending slightly beyond the perithecia, very thick (60–110 μ). Locules several, large (about 300 μ in diameter), irregular. Asci 4–8-spored, 85–102×7 μ long, slender, with long sterile base. Spore 1-celled, hyaline, oblong, 14–20×3.5 μ. Paraphyses filiform.

On *Ocotea leucoxylon*, Monte Alegrillo, 4725 (type), 732, 1347.

Entirely distinct from *Phyllachora ocoteicola*, although often upon the same leaf.

Catacauma palmicola, sp. nov. (figs. 10–12).—Stromata conspicuous above, few below, black, shining, oval, 1–6×1–3 mm.,

with rounded surface, scattered and separate or clustered and confluent. Diseased area extending through the leaf, brown below. Stroma developing between epidermis and palisade cells, often 300 μ thick. Locules irregular in shape, often 500–600 μ wide, basal layer hyaline, thin, lateral walls brown, thick; clypeus black, 60–100 μ thick. Asci numerous, 8-spored, saccate, thin-walled. Spores inordinate, cylindrical, 28–43×12–14 μ, hyaline, continuous.

On *Thrinax ponceana*, Vega Baja, 7716 (type).

CATACAUMA URBANIANUM (A. and H.) Th. and Syd. (fig. 13).

On *Calyptranthes Krugii:* El Yunque, 8243; Maricao, 3677, 3740.

What appears to be the same fungus, although usually hypophyllous and showing a concentric arrangement of stromata, occurs on an unknown Myrtaceous host, no. 5766, San German. Another specimen from Monte Alegrillo, 4526, shows the characteristic acervuli and spores, but is mainly conidial. The *Septoria*-like conidia are borne in very large cavities in the stromata. The ascospores in these specimens are slightly longer (17–20 μ) and slightly thinner (5 μ) than called for by description.

Catacaumella Gouaniae, sp. nov. (figs. 14, 15).—Mainly epiphyllous, rarely hypophyllous. Spots barely exceeding the stromata, hardly visible below. Stromata abundant, roughly circular, 2–3 mm. in diameter, raised, wrinkled, shining black, developing between the epidermis and the palisade cells and made up of parallel cells perpendicular to the leaf surface. Loculi large, flat, 500 μ wide, about 150–160 μ deep, single or few in each stroma. Ostiole very large and distinct. Asci thin-walled, irregular, 8-spored, 61–68×10–11 μ, inordinate. Spores hyaline, 1-celled, ovoid or pyriform, irregular, 14–20×10 μ. Paraphyses none.

On *Gouania polygana:* Mayaguez, 3923 (type), 1049; Salinas, 6798; Dos Bocas, 6007, 8092; Maricao, 8953; on *Gouania lupuloides*, Arecibo-Lares road, 7230.

The last specimen shows the stromata smaller and more abundant upon the lower surface than is the case with the other specimens.

Phaeodothopsis Eupatorii, sp. nov. (figs. 16, 17).—Spot not exceeding the clypeus. Stromata numerous, circular, 1–4 mm. in diameter, black, rough with perithecia, almost exclusively epiphyllous; developing first in the epidermis, producing an

extensive clypeus, then developing the stromata between this clypeus and the palisade cells. Loculi globular or lenticulate, 100–250 μ in diameter, 80 μ high, by pressure sometimes pushing into the mesophyll. Asci about 110×17 μ, cylindrical, 8-spored, inordinate. Spores 20×7 μ, 1-septate about one-third the distance from one end, brown when mature. Paraphyses filamentous, branching.

On *Eupatorium portoricense*, Dos Bocas below Utuado, 6866 (type), 6034, 6830, 6437, 6861, 6032, 6537.

The clypeus is strictly epidermal, and under it very numerous loculi develop, each with an ostiole reaching through the clypeus. The occasional pressing of the perithecia into the mesophyll sometimes gives this the appearance of closer relationship to the Phyllachorineae, but its relationship is clearly with the Scirrhiineae.

Halstedia, gen. nov.—Asci borne in a locule in a superficial stroma.

Type *H. Portoricensis*. Named in honor of BYRON D. HALSTED.

Halstedia portoricensis, sp. nov. (figs. 18, 19).—Stromata amphigenous but more abundant and larger above, densely black, 1–4 mm. in diameter, flat, with surface in the older parts corrugated, or sometimes raised in the center, strictly superficial, non-radiate. Perithecia up to 400 μ in diameter, 160 μ from base to top, internal measurements. Asci 8-spored, 68–85×14 μ, cylindrical. Spores oval, continuous, hyaline or pale straw-colored, 17×10 μ.

On *Sideroxylon foetidissimum*, Quebradillos, 9239 (type).

The fungus consists of a densely black stroma which in the center is nearly 200 μ in thickness, thinning at the edges to the thickness of the mycelium. The stroma is flat-topped, the bulging due to the development of the perithecium usually resulting in a downward thrust and displacement of the leaf rather than of the upper layer of the stroma (fig. 16). In some instances the reverse is true, with an upward bulging. Closest search failed to reveal any evidence of penetration of the fungus through the epidermis, or of any mycelium or signs of disease in any of the host cells. There is no ostiole, and the perithecium is poorly developed, if indeed it is more than a locule in the stroma. The fungus shows close kinship with the Dothideales, but cannot be placed in any of the families of that order as characterized by THEISSEN and SYDOW. It differs from typical Perisporiaceae in the absence of a clearly developed perithecium and in possessing a stroma. It forms an interesting transition form between these two groups, and may for the present be regarded as Perisporiaceous.

Perisporiales

Perisporiaceae

Dimerina monenses, sp. nov. (fig. 20).—Epiphyllous, rarely hypophyllous, diffuse over the leaf surface. Mycelium superficial, scant, dark, irregular, 3 μ thick with thinner side branches. No hyphopodia, perithecia rough, irregularly spherical, 45–60 μ in diameter, without ostiole, arranged in close clusters of 10 or more on a close dark subicle. Clusters 150–300 μ in diameter. Asci numerous, elliptical, 34×17 μ, obtuse, 8-spored. Spores inordinate, hyaline or very pale-smoky, 13–16×3 μ, obtuse, 2-celled.

On *Jacquinia barbasco*, Mona Island, 6087.

While the spores and asci agree well in size with those of *Dimerina eutricha* and *D. negeriana*, our species does not agree with these forms in other characters. Agreement as to asci and spores is close with *Asterina paupercula* E. and E., but our perithecium is not that of an *Asterina*.

Hysteriineae

Gloniella rubra, sp. nov. (fig. 21).—Perithecia oblong, scattered, numerous, epiphyllous, black, 600–1500×180–250 μ, opening by one or more longitudinal clefts; the perithecial contents thus exposed are red (near color no. 13 of Saccardo's scale). Asci long-cylindrical, very crooked, especially at the tip, 8-spored, 85–92×10 μ, inordinate. Paraphyses numerous, filiform, long. Spores hyaline or very faintly tinted, 1–3, mostly 3-septate, fusoid, 23–26×3 μ.

On *Arthrostylidium multispicatum* Pilg., El Alto de la Bandera, 4363 (type).

This species is somewhat like *G. pusilla* Sacc., but differs from it in its carbonaceous perithecium, red contents, curved asci, etc.

Pleosporaceae

Physalospora Hoyae, v. Hohn. (fig. 22).

On *Ficus*, Mona Island, 6234, 6169.

This very pretty form I refer with some hesitancy to the preceding species. The spores in my specimen are uniseriate, and are considerably narrower than the description of von. Hohnel calls for. *P. elasticae* Koord. is close kin, but differs in the rounded spores. Pycnidia are present, bearing slender filamentous spores, 7×1 μ.

Mycosphaerellaceae

Guignardia Justiciae, sp. nov. (figs. 23, 24).—Diseased spot indefinite, finally yellowish and pale, rather evenly beset with perithecia, 1–2 mm. distant from each other. Perithecium globose, completely imbedded in the leaf, 265 μ in diameter and depth, its wall dark, several cells (34 μ) thick. Host tissue surrounding the perithecium hypertrophied to a distance of about 125 μ in every direction from the perithecium. The resulting "gall" is visible from either side of the leaf, and has the superficial appearance of a stroma with a single central perithecium. The ostiole develops late. Paraphyses none. Asci clavate, usually with a long stipe; body of ascus 17–20×61 μ; total length, including stipe, 125 μ. Spores 8, inordinate, hyaline, 1-celled, oval, 9–10×18 μ.

On *Justicia verticillaris:* Maricao, 806 (type); El Yunque, 2839; El Gigante, 8557; El Alto de la Bandera, 9046.

This fungus is noteworthy on account of the peculiar gall-like formation surrounding each perithecium, the thick wall, and the peculiar long-stalked asci.

Guignardia Tetrazygiae, sp. nov.—Spots indefinite, irregular, 1–2 cm. in diameter or occupying the whole leaf, tan-colored, centers studded with the perithecia which are scattered evenly and profusely over the affected areas. Perithecia black, conspicuous both above and below, about 160 μ in diameter, thick-walled. Asci, sporiferous part oval, 45×27 μ, 8-spored, inordinate, stipe long, slender, 30–60×4–5 μ. Paraphyses none. Spores 1-celled, hyaline, oval, obtuse, 24×10 μ.

On *Tetrazygia* sp.: San German, 4567 (type); Vega Alta, 4148.

This differs from *Laestadia melastomalum* (Lev.) Sacc. in the absence of paraphyses, shape of asci, and other characters. The leaf spot is very characteristic.

Guignardia Nectandrae, sp. nov.—Spots indefinite when young, becoming definite as the host tissue dies, then angular, 2–6 mm. in diameter, showing from both sides of the leaf. Perithecia opening on both sides of the leaf, more abundant below, scattered, located in the mesophyll but causing swelling of both leaf surfaces. Perithecia thin-walled, pale, 70–85 μ in diameter,

located deep in the mesophyll. Asci clavate, 100–115×20 μ, 8-spored. Spores hyaline, oval, 21–24×8–10 μ, 2-celled, septa either in the middle or more frequently located near one end.

On *Nectandra coriacea* (?), Quebradillos, 4994 (type).

This fungus is of very distinctive appearance upon the leaves, where the erumpent perithecia so closely simulate a rust in appearance that the author was led to place it with the rusts on mere casual examination.

Sphaeriaceae

Zignoella algaphila, sp. nov.—Mycelium fine, pale to brown, twining around and penetrating its algal host and turning it brown. Perithecia black, 90×170–180 μ, variously formed but usually bottle-shaped, broadest a little above the base, with a prominent beak about 24 μ in diameter and with the fibers arranged parallel around the ostiole. Surface coarsely reticulate but not hairy; basal portion appearing as though hairy due to adhering remnants of mycelium. Asci numerous, 8-spored, cylindrical, 71×7 μ. Paraphyses fine, threadlike. Spores hyaline, 3-septate, pointed at each end, 17–21×3.5 μ.

On *Cephaleuros virescens* on *Artocarpus incisa*, Mayaguez, 51 (type).

The parasitic alga when alone on this host is yellow or often nearly colorless, but when invaded by the *Zignoella* all the colonies take on a dark hue, giving the whole leaf much the appearance of being mildly affected with sooty mold. The genus *Zignoella* is large and composed mainly of wood-inhabiting saprophytes. One is listed on *Valsa*, one on the thallus of *Castagnia*, and two species (*Z. enormis* Pat. and *Z. cubensis* H. and Pat.) on the alga *Stypocaule*. These thallus-inhabiting forms, however, are markedly different from the present species.

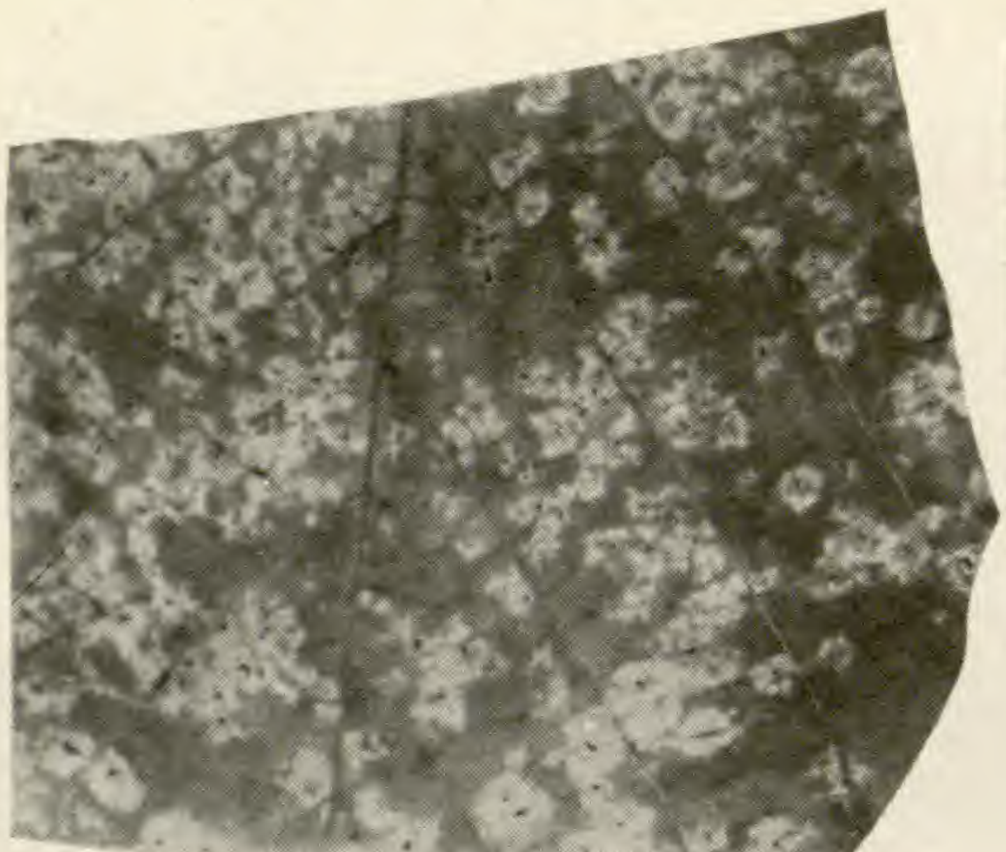
Sphaeropsoidales

Phyllosticta bonduc, sp. nov.—Spots indefinite, large, starting usually at edge or apex and progressing over the whole leaflet. Pycnidia numerous, black, scattered, ostiolate, about 160–190 μ in diameter. Wall about 17 μ thick, ostiole large, irregular. Conidiophores simple, hyaline, arising from sides and base of the pycnidium. Conidia hyaline, 1-celled, oblong, 21×4 μ, somewhat irregular in shape.

On *Caesalpinia bonduc*, Guanica, 360 (type).

This fungus is quite distinct from *Phyllosticta guanicensis*.

University of Illinois
Urbana, Illinois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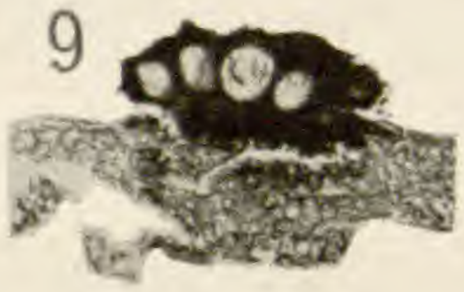
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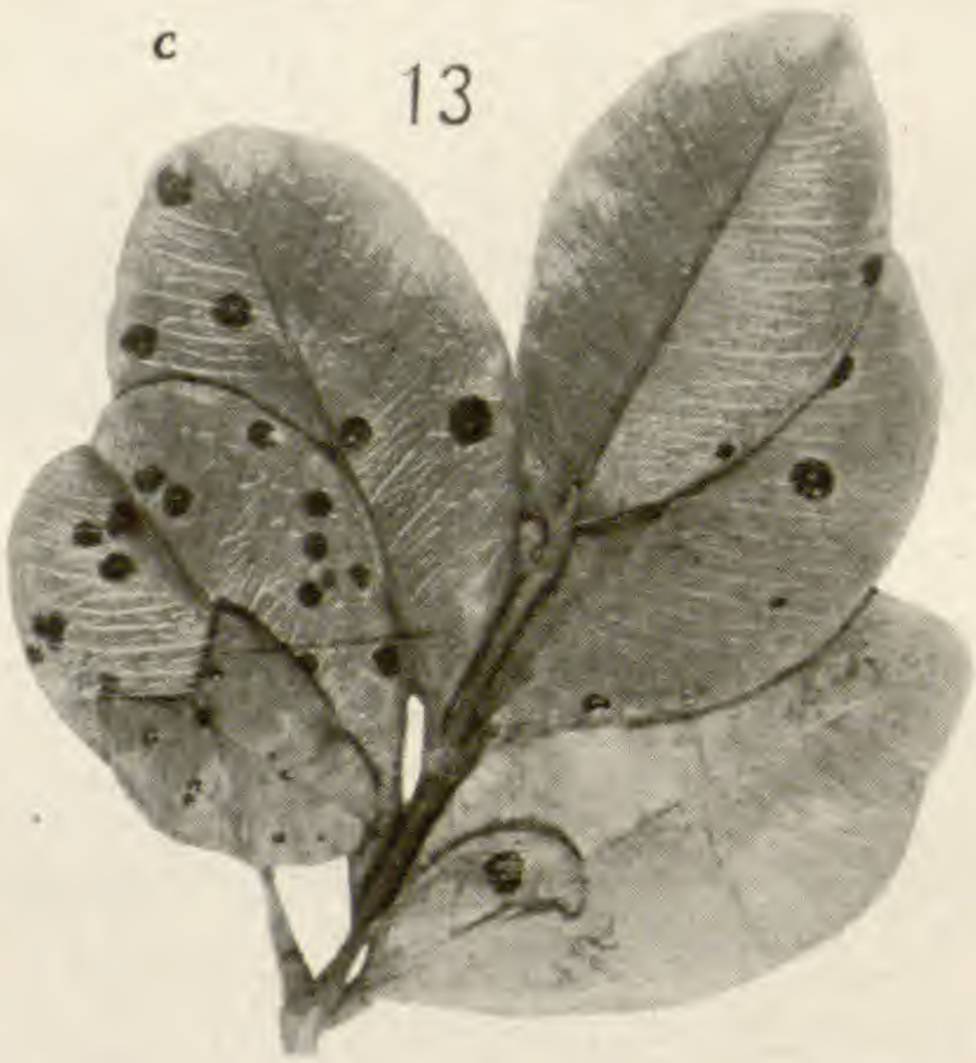
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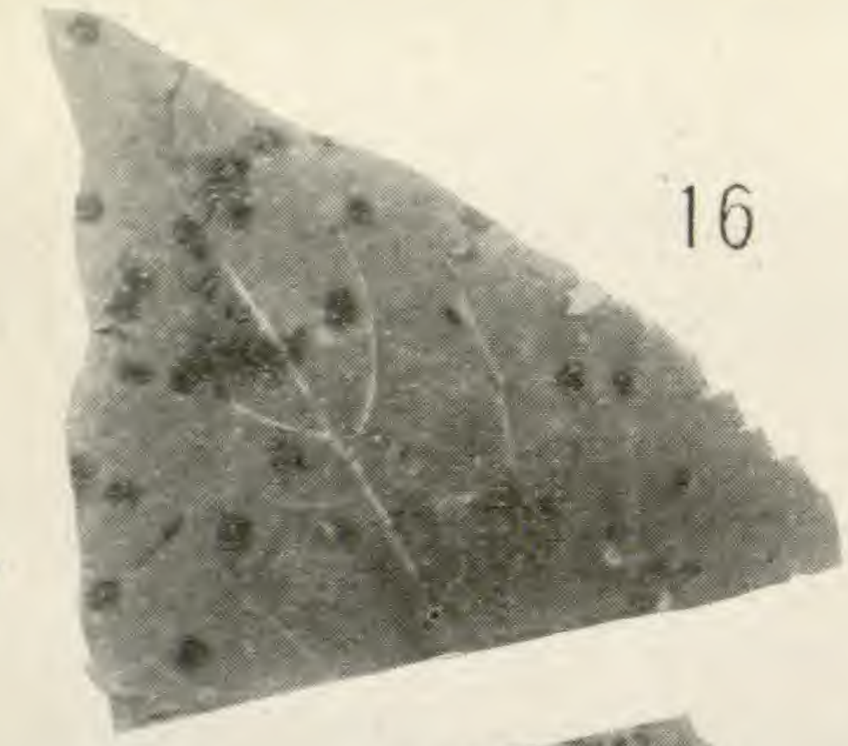
STEVENS on FUNGI



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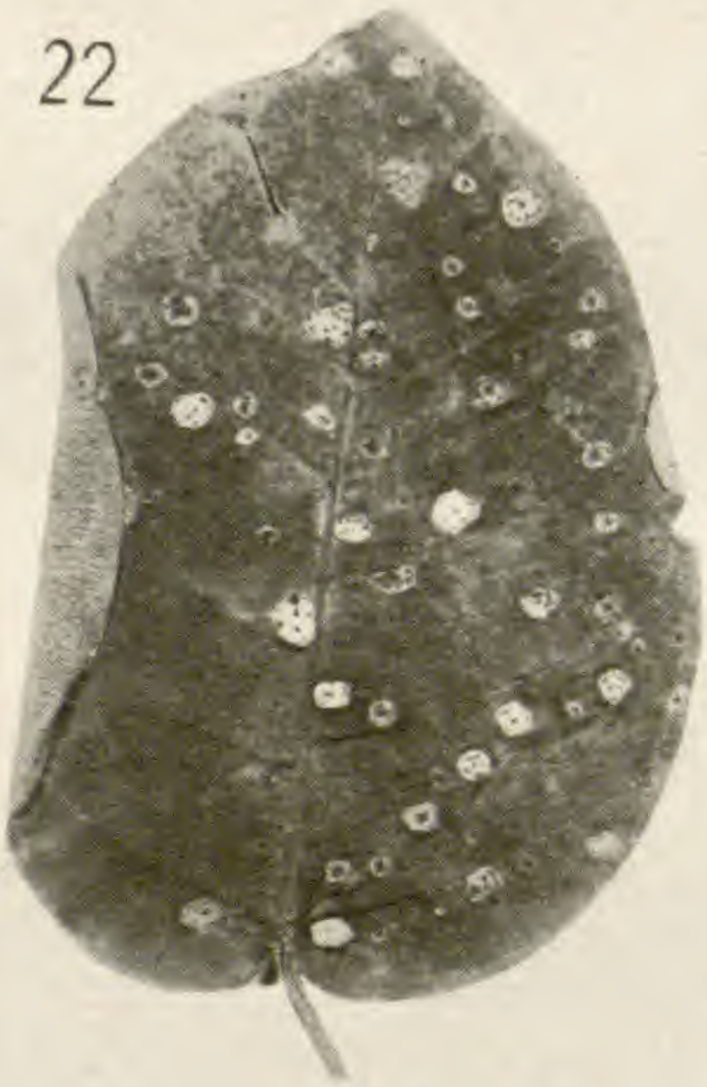

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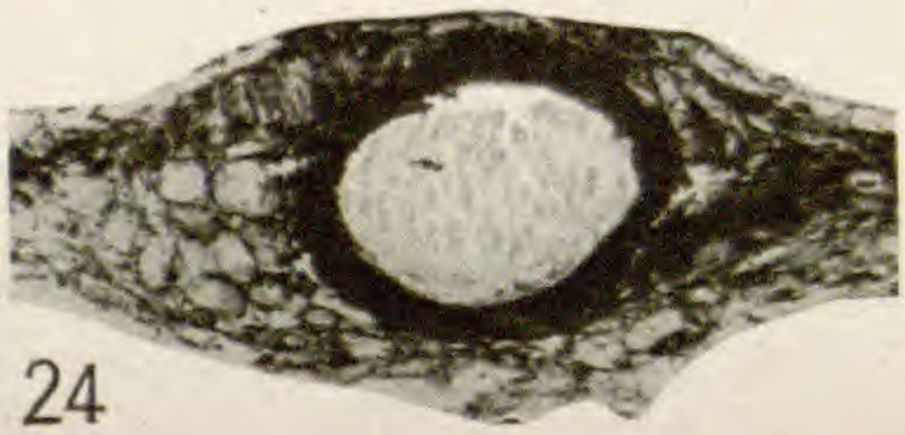

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STEVENS on FUNGI

EXPLANATION OF PLATES XIII, XIV

FIGS. 4, 5.—*Auerswaldia Cecropiae* P. Henn.: fig. 4, habit, showing, *a*, abundant scattered young spots (no. 361); *b*, older infections each surrounded by discolored spot (no. 9043); *c*, still older spots with much dead tissue (no. 9043); fig. 5, old, well-developed stroma completely occupying leaf from epidermis to epidermis.

FIGS. 6, 7.—*Uleodothis Pteridis*, sp. nov.: fig. 6, habit; fig. 7, stromata occupying whole mesophyll with locules on both surfaces.

FIGS. 8, 9.—*Dothidella portoricensis*, sp. nov.: fig. 8, habit, a leaf segment (no. ×62); fig. 9, cross-section of stroma.

FIGS. 10–12.—*Catacauma palmicola*, sp. nov.: fig. 10, habit; numerous stromata on piece of palm leaf; fig. 11, young stroma and locules, showing that it is strictly subcuticular; fig. 12, sectional view of mature stroma showing 3 locules.

FIG. 13.—*Catacauma urbanianum* (A. and H.) Th. and Syd., showing habit, no. 3577.

FIG. 14.—*Catacaumella Gouaniae*, sp. nov.: habit, stromata scattered over leaf.

FIG. 15.—*Catacaumella Gouaniae*, sp. nov.: stroma in section, showing that it is formed entirely above palisade cells.

FIGS. 16, 17.—*Phaeodothiopsis eupatorii*, sp. nov.: fig. 16, habit, showing stromata of different ages (no. 6866); fig. 17, stroma in cross-section.

FIGS. 18, 19.—*Halstedia portoricensis*, sp. nov.: fig. 18, general view of stromata on leaf; fig. 19, stroma in section, showing depression of leaf by growth of stroma.

FIG. 20.—*Dimerina monensis*, sp. nov., showing habit.

FIG. 21.—*Gloniella rubra*, sp. nov., showing habit.

FIG. 22.—*Physalospora Hoyae* v. Hohn., showing habit.

FIGS. 23, 24.—*Guignardia Justiciae*, sp. nov.: fig. 23, habit; fig. 24, section through hypertrophied portion showing perithecium.

SPERMATOGENESIS IN BLASIA

LESTER W. SHARP

WITH PLATE XV

Introduction

The following brief account of spermatogenesis in *Blasia pusilla* is based upon preparations made from a limited amount of material collected near Chicago several years ago. The preparations, which were originally made for use in classes, proved upon careful examination to show with admirable clearness all stages included in the last spermatogenous mitosis and the transformation of the androcyte (spermatid) into the spermatozoid. Since the results of the examination differ in two important points from those reported by WOODBURN (12) in the only previous paper dealing with these features in *Blasia*, they are here recorded.

Description

The description will begin with the spermatogenous cells of the penultimate generation, the androcyte mother cells, to use the terminology of ALLEN (1). The cells of the earlier generations (androgones) have been examined, and nothing which it is safe to call centrosomes has been observed. Unfortunately, however, the material did not show many androgone nuclei in division; anaphases were present, but metaphases, where centrosomes are usually most conspicuous if present at all, were not found. No conclusive statement can be made, therefore, regarding the presence or absence of centrosomes in the androgones.

In the androcyte mother cell, before the stage represented in fig. 1, the cytoplasm has an almost homogeneous appearance, and included in it are several granules or vaguely defined areas. In some cells these granules, from 1 to 6 or more in a thin section, may appear to be all alike; while in other cells one or two of them may be more sharply defined and more deeply stained than the others. It is possible that of these several granules two survive as the

centrosomes shown in fig. 1, after the manner of the "black granules" in the body cell of *Dioon* (CHAMBERLAIN 4). On the other hand, it would be possible to select a series of cells illustrating the divergence of daughter centrosomes arising by the division of one, as in *Equisetum* (SHARP 8); or even to show the origin of the bodies in question from the nucleus, as described by WILSON (10) for *Atrichum* and *Mnium*. The writer, however, believes that the evidence afforded by his material is insufficient to support any of these hypotheses in the case of *Blasia*. The present description, therefore, will begin with a stage (fig. 1) at which the identity of the centrosomes is unmistakable, the question of their origin and earlier history being left an open one.

Two centrosomes, whatever may be their previous relation to other cell granules, soon stand out with great distinctness as intensely staining bodies near the cell membrane at opposite poles of the androcyte mother cell (fig. 1). At this time the cell is still rather square in section, since it has only begun to round off from its neighbors, and the centrosomes commonly occupy the corners, as shown in the figure. From each centrosome a conical group of very faint fibers extends toward the nucleus, which is somewhat flattened on the sides facing the centrosomes. While the nucleus is undergoing the prophasic changes (fig. 2) these fibers become more plainly visible, and when the nuclear membrane disappears they become attached to the chromosomes and establish the achromatic figure with the centrosomes at its poles.

It is at metaphase that the spindle is seen most clearly (fig. 3). As noted by WOODBURN (12), it may lie either straight or obliquely in the cell. Furthermore, the cells may round up and alter considerably in shape while mitosis is in progress, so that although the centrosomes may at first be situated near the corners of the cell, all appearance of the diagonal division so characteristic of many bryophytes may in many cases be lost by the time the metaphase and succeeding stages are reached (figs. 4, 5).

When the chromosomes reach the poles at the end of the anaphase (fig. 4), they usually come in contact with the centrosomes. As a result the latter, which are very minute, are often difficult to find at this stage. Careful search, however, reveals cells in which

they stand out clearly a little apart from the chromosome groups. From this time onward they become increasingly distinct. As the membranes form about the reorganizing daughter nuclei at telophase the centrosomes are left just outside in the cytoplasm (fig. 5), and while cell division is being completed they move away from the nucleus and take up positions nearer the cell membrane (fig. 6).

The two androcytes (spermatids), between which no cell wall is laid down, quickly round off from each other (fig. 7). In probably the majority of cases they are somewhat triangular in shape, owing to the usual diagonal plane of the division which differentiates them. In each androcyte the blepharoplast, as we may call the centrosome in view of the function it performs in the cell which it now occupies, enlarges considerably and becomes somewhat elongated.

A careful search has been made in the cytoplasm of the androcytes for accessory structures corresponding to the "chromatoider Nebenkörper" (IKENO 6) or "limosphere" (WILSON 10), the "percnosome" and the "apical body" (ALLEN 2) described by other investigators of bryophyte spermatogenesis; but, as WOODBURN (12) also reports, nothing which can confidently be regarded as such a body has been found. Occasionally there is observed in the cytoplasm a darker area, which, although it is as a rule rather vague in outline (fig. 7, below and at left of nucleus in each cell), may in certain cases be more definitely delimited (fig. 9). A similar appearance is also often seen in the later stages of spermatogenesis (figs. 15, 16, 18, 19). It may well be that we are dealing here with a limosphere or other accessory body, but without more trustworthy evidence for its constant presence and regularity in behavior, at present it does not seem advisable to attribute to this body any special significance in the case of *Blasia*. The cytoplasm of the androcyte frequently contains a large vacuole, which may or may not lie near the blepharoplast (fig. 8).

The blepharoplast now begins to undergo a series of transformations which ultimately result in the formation of the cilia-bearing thread of the spermatozoid. After elongating very slightly, as previously noted, the blepharoplast becomes constricted

(fig. 10, upper cell) and divides by a process of simple fission into two portions (fig. 10, lower cell). These two portions, or blepharoplast granules as they may be termed, often lie very close together, but in many cases they are so far apart that there can be no doubt that the fission is complete. As a rule one of the granules at once begins to elongate, while the other remains relatively unchanged, so that many cells show two bodies, one of them round and the other comma-shaped, lying close together near the cell membrane (fig. 11). At about this stage the granules usually move closer to the nucleus. The comma-shaped granule continues to elongate (fig. 12) and divides again; whether the other granule also divides or not is a difficult matter to determine. The granules continue to multiply by fission (fig. 13) until several are present in a row (figs. 14, 15); seven was the largest number counted with certainty. The granules now appear less distinct from one another; it seems that they gradually undergo a coalescence (figs. 14–16), but it may also be that some of the fissions are incomplete, some of the granules therefore never being entirely separate.

The nucleus at this time moves more closely against the beaded blepharoplast (fig. 15) and begins to draw out into a point by the side of the latter (fig. 16). Both nucleus and blepharoplast continue to elongate spirally, the association between them becoming constantly more intimate (fig. 17). Fig. 18 represents a cell like that of fig. 17 viewed from the direction indicated by the arrow; it is here seen that the blepharoplast is applied along one edge of the flattened point of the nucleus. As the transformation continues the boundary between nucleus and blepharoplast gradually becomes indistinguishable (fig. 19). Even at this late stage the irregular outline of the blepharoplast is still evident; the blepharoplast granules have not yet become so completely coalesced that the thread which they form is smooth in outline. The nucleus continues to elongate and condense, becoming increasingly slender, while two cilia grow out from the blepharoplast, which projects beyond the nucleus at the anterior end. The spermatozoid is now mature (fig. 20) and ready to escape from the antheridium.

Discussion

The two main points wherein this description disagrees with that of WOODBURN (12) are as follows. First, according to that author there are no indications of centrosomes in the spermatogenous mitoses, the blepharoplast first appearing as a cytoplasmic differentiation in the androcyte. On the contrary, the present writer finds that centrosomes are present at all stages of the last mitosis, and that these persist as the blepharoplasts of the androcytes. Second, WOODBURN states that the blepharoplast in the androcyte undergoes a simple elongation to form the cilia-bearing thread, whereas the present writer sees it fragmenting to several pieces which coalesce to form the thread somewhat after the manner of the blepharoplasts of *Equisetum* and *Marsilia* (SHARP 8, 9).

It is not improbable that this disagreement is due in part to actual differences in the two lots of material studied. Although the single species of the genus, *Blasia pusilla*, was used in both instances, a comparison will show that the cells described in the present account are little more than half the size of those figured by WOODBURN. Although it is possible, therefore, that the two lots of material represent two varieties, too much weight should not be placed upon a size difference, for it is known in certain cases (*Equisetum*, SHARP 8) that androcytes and spermatozoids often vary considerably in size in the same lot of material.

Lack of agreement as to the presence of centrosomes during mitosis is perhaps not surprising. Because of their extreme minuteness the centrosomes might easily be overlooked in the stages previous to that at which WOODBURN first finds them, and at which they enlarge and become really conspicuous for the first time. With regard to the fragmentation of the blepharoplast, on the other hand, it is more difficult to understand why material actually the same should be interpreted so differently. In the writer's material the process of fragmentation is shown with great clearness; only occasionally is anything found in good preparations which might be interpreted as a uniformly elongating blepharoplast. Moreover, in no case has a condition approaching that shown in WOODBURN'S fig. 11 been observed. The nucleus becomes closely applied to the blepharoplast when the latter is in

the form of a short lumpy rod or series of granules, and at no time does the blepharoplast have the form of a long slender thread free from the nucleus as in WOODBURN'S figure. The writer, therefore, is inclined to attribute the disagreement for the most part to actual differences in the material studied rather than to differences in interpretation.

The phenomenon of fragmentation is probably the most interesting feature of the blepharoplast of *Blasia*. In all previous accounts of bryophyte spermatogenesis, including those of IKENO (6) on *Marchantia*, WILSON (10) on *Pellia*, *Polytrichum*, and *Atrichum*, WOODBURN (11, 12, 13) on several liverworts and *Mnium*, Miss BLACK (3) on *Riccia*, and ALLEN (2) on *Polytrichum*, the blepharoplast is reported to elongate without breaking up into smaller portions. ALLEN (2) states that "while the possibility of a somewhat similar occurrence [fragmentation] is suggested by the rather knotty appearance of the blepharoplast of *Polytrichum* when it begins to elongate, there is no time when it is visibly resolved into smaller bodies." In *Blasia*, therefore, we have the only known instance in bryophytes of such a fragmentation of the blepharoplast as occurs in *Equisetum*, *Marsilia*, and the cycads.

Although fragmentation is in general a characteristic of the blepharoplasts of the cycads, and only occasionally found in pteridophytes (*Equisetum* and *Marsilia*), it is now evident that it may occur in forms lower in the scale. Moreover, it is seen that it is not, as might be supposed, merely a means by which large blepharoplasts become transformed, for the blepharoplasts of *Equisetum* and *Marsilia*, and especially those of *Blasia*, are very small. Although the details of the process of fragmentation differ in the various cases (by simple fission in *Blasia* and by vacuolization in the other forms), it is scarcely to be doubted that the phenomenon is a result of similar causes in all. In attempting to find a possible historical reason for it, one is struck by the resemblance between the fission of the blepharoplast in *Blasia* (fig. 10) and the division of an ordinary centrosome before mitosis. If the blepharoplast actually represents a centrosome, as the writer (8) believes the evidence indicates, it is at least possible that its frequent fragmentation, in spite of the fact that in the more advanced

forms (cycads) this fragmentation becomes a very much modified process, may be a manifestation of the power of division which is one of the chief characteristics of centrosomes. According to this interpretation the first fission of the blepharoplast of *Blasia* (fig. 10) would correspond to the centrosome division which would normally occur if another mitosis were to take place, and the further fragmentation would represent a further manifestation of the centrosome's power of division which may have been retained from a time when more spermatozoids were produced from a mother cell, and which has in some way become a feature of the development of the cilia-bearing structures. In this way *Blasia* may shed light upon the origin of the remarkable behavior of the cycad blepharoplasts.

To this idea, which presents itself as a suggestion and may scarcely deserve to be proposed as a theory, there are obviously many objections. Chief among these is the fact that fragmentation is most conspicuous in the blepharoplasts of the cycads, but developed almost not at all in those of the bryophytes, which would be expected to have retained in the manner of their elongation more evidences of a derivation from normal centrosome division. It is possible, however, that the simple fission of the blepharoplast as seen in *Blasia* was soon replaced in most bryophytes and pteridophytes by uniform elongation without fragmentation through the failure of the fission to occur after the slight elongation normally preceding it (figs. 7–9), this elongation then continuing to form the uniform cilia-bearing thread. Fragmentation would thus be a retained feature in *Blasia*, *Equisetum*, *Marsilia*, and the cycads, although the manner in which it is accomplished in the higher forms (through a complex process of vacuolization rather than simple fission) would still be regarded as an advanced feature subsequently evolved. Whether, therefore, the objection stated rules out the suggested explanation or not can scarcely be decided in view of the fact that the evidence at hand has been obtained from so few bryophytes and pteridophytes, comparatively speaking, and especially in view of our lack of adequate knowledge of blepharoplast origin and behavior in the algae.

A further objection may be seen in the case of animal spermatogenesis, in which an undoubted centrosome elongates without fragmentation as it performs its rôle in the development of the motor structures. It is noteworthy, however, that cilia are frequently seen growing from recently divided centrosomes in the case of certain insect spermatocytes (HENNEGUY 5) in much the same fashion that the cilia start to grow from the recently formed blepharoplast granules in *Equisetum* (SHARP 8). Moreover, in the Flagellata, which should furnish evidence more valuable than that in the higher animals, it is known that in certain cases blepharoplasts arise from functional centrosomes by division (see MINCHIN 7, pp. 82 ff.).

Although there is thus seen to be considerable evidence for the derivation of blepharoplast fragmentation from normal centrosome division, this evidence is probably best regarded as scarcely sufficient to warrant the establishment of such an interpretation as a general theory.

The question of the relation of the centrosome to the blepharoplast has been fully discussed by the writer in his papers on *Equisetum* and *Marsilia* (8, 9). It will be sufficient here to recall that the conclusions were reached that the blepharoplasts of bryophytes, pteridophytes, and gymnosperms are "ontogenetically or phylogenetically centrosomes" (IKENO); that these centrosomes become more and more restricted in the life history in passing upward through these groups; that they are retained in spermatogenous cells because of the biological importance of the cilia-bearing function which they there perform; and that in connection with this function they have become profoundly modified, losing many of the characteristics of centrosomes and assuming new characteristics not exhibited by centrosomes elsewhere.

To these conclusions *Blasia* furnishes support of no new kind; it merely confirms them by affording another example of blepharoplasts arising from centrosomes functional in mitosis. How extensive this centrosome behavior is in the case of *Blasia* the present study may not show, for, as stated in the description, the writer's material does not enable him to say whether the bodies in question arise from preexisting ones by division or not, or whether they

are present at only one or more than one spermatogenous mitosis. So far as actual evidence goes, it is possible to state unreservedly only that they are present from the stage represented in fig. 1 onward, and that through the single mitosis they appear to perform the usual functions of centrosomes. The discovery of fragmentation in the blepharoplast of a bryophyte serves to confirm the view that the blepharoplasts of all groups above the algae are homologous structures, and the details of the process aid materially in accounting for the behavior of those blepharoplasts which have become least centrosome-like.

Summary

1. Centrosomes are present in *Blasia* at all stages of the mitosis which differentiates the androcytes, and in the androcytes they persist and function as the blepharoplasts.

2. In the transformation of the androcyte into the spermatozoid, the blepharoplast fragments repeatedly by simple fission, forming a number of distinct granules which coalesce to form a short lumpy rod. This rod elongates and becomes a more uniform thread bearing two cilia, while the nucleus also elongates in intimate union with it to form the body of the spermatozoid. The present instance is the first in which blepharoplast fragmentation has been reported in a bryophyte.

3. It is possible that the fission of the *Blasia* blepharoplast, and therefore the more complex fragmentation of the blepharoplasts of *Equisetum*, *Marsilia*, and the cycads, may be homologized with the normal division exhibited by ordinary centrosomes.

CORNELL UNIVERSITY

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EXPLANATION OF PLATE XV

All figures were drawn at the level of the table with the aid of an Abbé camera lucida from cells stained with iron alum-haematoxylin. Examination of the cells was made under a Zeiss 2 mm. apochromatic objective, N.A. 1.40, but because of its slightly greater magnifying power a Spencer 2 mm. achromatic objective was used with an 18 ocular for outlining the drawings. The figures, which have not been reduced in reproduction, show a magnification of 4200 diameters.

FIG. 1.—Androcyte mother cell (penultimate spermatogenous cell) with two centrosomes.

FIG. 2.—Prophase of last spermatogenous mitosis; centrosomes at poles of developing spindle.

FIG. 3.—Metaphase; centrosomes at spindle poles.

FIG. 4.—Late anaphase; centrosomes present.

FIG. 5.—Telophase; centrosomes near daughter nuclei.

FIG. 6.—Late telophase; each cell has one centrosome (blephoraplast).

FIG. 7.—Androcytes (spermatids) rounded off; blepharoplast slightly elongated in each; dark body near nucleus.

FIG. 8.—Pair of androcytes with vacuoles in cytoplasm.

FIG. 9.—Androcyte with dark body (limosphere?) in addition to blepharoplast.

FIG. 10.—Pair of androcytes: blepharoplast undergoing fission in upper cell; two blepharoplast granules resulted from fission in lower cell.

FIG. 11.—Pair of androcytes showing elongation of one blepharoplast granule.

FIG. 12.—Androcyte; slightly later stage.

FIG. 13.—Blepharoplast granules multiplying.

FIG. 14.—Later stage; granules somewhat coalesced.

FIG. 15.—Nucleus moving against blepharoplast.

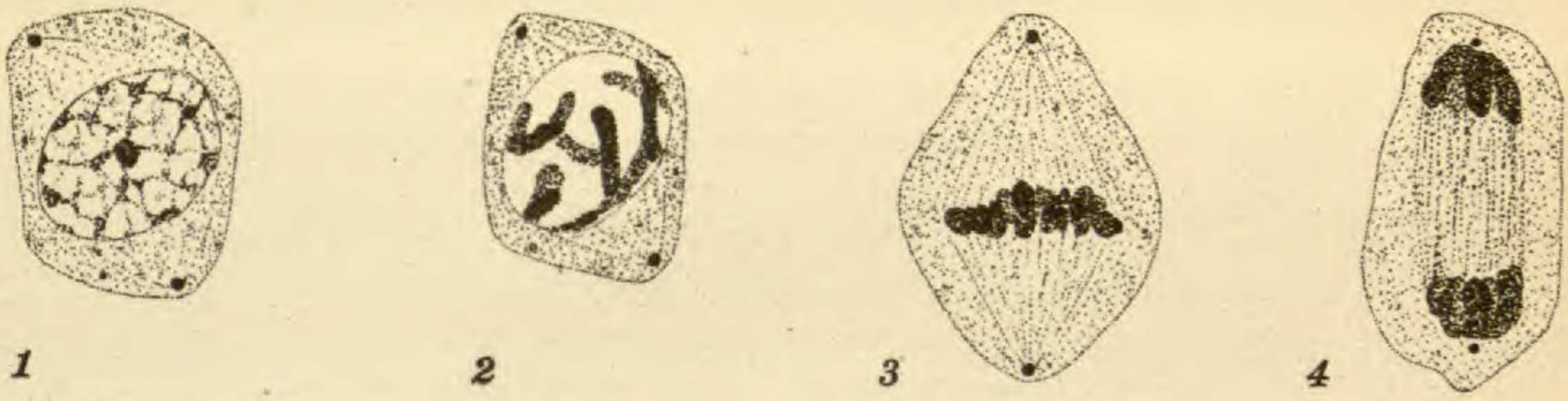
FIG. 16.—Nucleus elongating by side of blepharoplast; blepharoplast granules becoming coalesced.

FIG. 17.—Later stage; blepharoplast and nucleus becoming closely associated.

FIG. 18.—Cell like that of fig. 17 viewed from direction indicated by arrow; blepharoplast lying along edge of flattened point of nucleus.

FIG. 19.—Later stage; blepharoplast still irregular in outline; boundary between nucleus and blepharoplast indistinguishable.

FIG. 20.—Mature spermatozoid ready to escape from antheridium.



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L. W. Sharp, del.

SHARP on BLASIA

CURRENT LITERATURE

NOTES FOR STUDENTS

Weather and fruitfulness.—DORSEY[1] has done much to place on an experimental basis a subject concerning which there have been many erroneous popular beliefs. In so far as it affects pollination and fertilization, he divides weather into 4 components, rain, temperature, wind, and sunshine. Wind and sunshine in themselves are of minor importance. Rain prevents the dehiscence of the anthers, or causes them to close if they have already dehisced. While this is beneficial in retaining much of the pollen in the anther during a rain, the pollen is not available for pollination during this time. Contrary to popular belief, rain does not cause the pollen to burst, and although the stigmatic fluid may be diluted thereby, this does not seem to be injurious. Some pollen may be washed from the stigma by rain, but an abundance is left for fertilization. Rain does not injure the viability of pollen. Low temperatures retard the growth of the pollen tube, but do not seem to cause delay in the abscission of the style. The stigma is receptive for 4–6 days and then rapidly disintegrates. The style abscisses 8–12 days after bloom. A delay in pollination due to rain, or slow pollen tube growth due to low temperatures, may therefore eliminate fertilization by preventing the pollen tube from passing the point of abscission before the abscission of the style. Applying this analysis of weather to certain years of fruitfulness and to certain other years of non-fruitfulness, it is found that each year there is a definite correlation between the weather and the setting of fruit. The experiments are thus given a practical test.—S. V. EATON.

Determination of biological fluids.—Duggar and Dodge,[2] after discussing some of the difficulties encountered in examining biological fluids, particularly colored plant juices, by the indicator method of H ion determination, describe a new method which they have found satisfactory for the examination of colored plant juices. "The method consisted in simply arranging for each side of the colorimeter a pair of cups slipping to a certain depth one into the other. The method of procedure is then as follows. For the lefthand

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[1] DORSEY, M. J., Relation of weather to fruitfulness in the plum. Jour. Agric. Res. **17**:103–126. *pls. 13–15. fig. 1.* 1919.

[2] DUGGAR, B. M., and DODGE, C. W., The use of the colorimeter in the indicator method of H ion determination with biological fluids. Ann. Mo. Bot. Gard. **6**:61–70. 1919.

set, or column, water (or colored standard solution) is used in the outer cup, and the colored test fluid plus the indicator in the inner cup. After adjustment, this set is not removed from the colorimeter during an observation. In the case of the righthand set the outer cup contains the colored test fluid, while the inner cup is for the standard solution plus indicator. This set is placed on the right for convenience, as it may be necessary to compare with the test fluid a series of standards until an exact match is obtained. A rough comparison, of course, is made before selecting the standard solution for comparison. In each case the column must contain an equal depth of colored test solution and of standard or colorless liquid, the indicator being in the standard in the one case and in the test solution in the other. There are no optical difficulties, and unless the indicator combines with the test solution, the comparison may be perfect."

The authors believe this method is as rapid as and more accurate than other methods.—J. WOODARD.

Storied structure of dicotyledonous woods.—A recent paper by Record[3] continues his studies upon the storied or tierlike structure of woods. He finds this arrangement of the secondary elements characteristic of many dicotyledonous woods, occurring through a wide range of orders and families. Such woods on longitudinal section (particularly the tangential) present fine cross lines or striations ("ripple marks"), which may be due to (1) the horizontal seriation of the medullary rays, (2) the tierlike arrangement of the tracheids, wood fibers, vessel segments, and the secondary phloem elements, or (3) a combination of (1) and (2). In some woods the pit areas on the fibers are also in seriation. This storied structure has been found fairly characteristic of the families Leguminosae (40 genera), Bignoniaceae (3), Bombacaceae (3), Compositae (3), Malvaceae (4), Sterculiaceae (7), Tiliaceae (5), and Zygophyllaceae (3); and occurs in one or two genera of each of the following families: Amarantaceae, Ebenaceae, Hippocastanaceae, Moraceae, Sapindaceae, and Ulmaceae.

Particular attention has been given in the present investigation to the various elements storied, the uniformity and distinctness of these transverse lines (ripple marks), and the height of the tiers in each wood examined. "Ripple marks" are sufficiently constant in stems of considerable thickness to serve, the author believes, as a "valuable diagnostic feature."—LADEMA M. LANGDON.

Antarctic and sub-antarctic vegetation.—TURRILL[4] has embodied in a convenient and useful summary the botanical results of the Swedish expedi-

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[3] RECORD, S. J., Storied or tierlike structures of certain dicotyledonous woods. Bull. Torr. Bot. Club **46**:253–273. 1919.

[4] TURRILL, W. B., Botanical results of Swedish South American and antarctic expeditions. Roy. Bot. Gard. Kew Bull. 268–279. 1919.

tions to the antarctic regions. These have appeared from time to time in the report of SKOTTSBERG and others, and many have been noted in this journal.[5] The more recent reports have contributed to our knowledge of the vegetation of the portion of South America and adjacent islands between 48° and 56° S. Here is a rain forest covering a limited area, and composed of trees of low stature, few exceeding 10 m. in height. The conspicuous species include *Nothofagus betuloides*, *Drimys Winteri*, *Pseudopanax laetevirens*, and *Libocedrus tetragona*, the only conifer reaching Fuegia. In unforested areas dwarf shrubs, many from the heath family, and cushion plants are conspicuous.

Farther to the north the Valdivian rain forest occupies the region between the coast and the Andes, forming in the lower passes of the mountains a transition to the deciduous forest of the east slope. Between 41° and 44° S. a forest of *Libocedrus chilensis* is interposed between the rain forest and the deciduous. Many other formations are characterized, such as the pampas area east of the Andes, the alpine heaths and meadows, the tussock grass and the tundras. The bibliography includes 23 articles.—GEO. D. FULLER.

Influence of environment on form and structure.—FOLSAM[6] reports a study of the effects of 5 different degrees of soil water supply upon the structural features of *Ranunculus sceleratus* and *R. abortivus*. Plants were grown as pot cultures in a greenhouse. Water was supplied in amounts varying from complete submergence of soil and plant, to only enough soil moisture to support life. In the 24 which were studied 6 structural characteristics of *R. sceleratus* gave consistently larger values with progressively greater water supply in the first generation of plants. In the second generation, 2 of the 6 characters continued to show the same relation. They were (1) thickness of stem cortex, and (2) thickness of stem aerenchyma, both absolute and relative to cortex thickness. In the first generation 5 structural features of *R. abortivus* were found to be related in the same way to water supply. Of these the one relation of increased laminar area of root leaves with increased water supply was shown, although less consistently in the case of *R. sceleratus*. A third generation of the latter species was grown to determine whether the conditions of water supply of parent affected the laminar area of root leaves of progeny grown both as xerophytes and as amphibians. Seeds for this generation were obtained from the xerophyte group of the first generation, and from the amphibious group of the second generation. Progeny grown with a large water supply gave consistently increased laminar area of root leaves over plants grown with a small water supply, regardless of water relations of parents.—J. M. ARTHUR.

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[5] BOT. GAZ. **58**:96–98, 190. 1914; **63**:423. 1917.

[6] FOLSAM, DONALD, The influence of certain environmental conditions, especially water supply, upon form and structure in *Ranunculus*. Physiol. Res. **2**:209–276. 1918.

Osmotic pressure in the potato.—In an effort to throw some light on the physiological basis of tip-burn, LUTMAN[7] has studied the osmotic pressure of the potato plant throughout a growing season. In the young plant, when the foliage is being formed, the osmotic pressure of the leaves is greater than that of the stems. After the flower buds are formed and the tubers begin to grow, the stalks predominate over the leaves in osmotic pressure. Sugars account for the high pressures of the stalks. Tip-burn begins to appear at this stage. This higher osmotic pressure of the stalks is maintained during the hot weather of July and August. With the coming of cool rainy weather in September and the resumption of growth of the foliage, the leaves again gain the ascendancy. As the plant dies the osmotic pressure decreases, the soluble materials being largely transported to the tubers. The osmotic pressure of the growing tubers is always lower than that of the stems and leaves, although above that of the roots. The study does not explain tip-burn, although the author sees two possible explanations of it: (1) the loss of water from the leaves to the stems, due to the higher osmotic pressure of the latter; and (2) the lack of nourishment of the leaves, due to the translocation of food materials from the leaves to the tubers. The author draws some other theoretical and practical conclusions from his data.—S. V. EATON.

Anatomy of prairie plants.—Selecting the dominant species from some prairie associations, Miss HAYDEN[8] has studied their leaf structure and presented considerable data, the most valuable being in the form of plates from drawings of cross-sections. She concludes that prairie plants show a xerophytic tendency in their leaf structure in the form of specialized palisade tissue, thick-walled epidermis, the presence of water-storing tissue, and sometimes of trichomes.

In studying the subterranean parts of plants from the same habitats, including a larger number from swampy areas, the same author[9] again presents many data in the form of drawings. Her principal conclusions are that in a dry habitat there is a tendency to the production of prominent mechanical tissue and reduction of parenchymatous tissue. In moist habitats, however, parenchymatous tissue is well developed and aerenchyma is abundant in swamp plants. The subterranean stem is predominant in moist lowland regions, and is more efficient than roots in propagation.—GEO. D. FULLER.

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[7] LUTMAN, B. F., Osmotic pressures in the potato plant at various stages of growth. Amer. Jour. Bot. **6**:181–202. *figs. 2.* 1919.

[8] HAYDEN, ADA, The ecological foliar anatomy of some plants of a prairie province in central Iowa. Amer. Jour. Bot. **6**:69–85. *pls. 10–14.* 1919.

[9] ———, The ecological subterranean anatomy of some plants of a prairie province in central Iowa. Amer. Jour. Bot. **6**:87–105. *pls. 15–28.* 1919.

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RIPENING OF PEARS AND APPLES AS MODIFIED BY EXTREME TEMPERATURES

E. L. OVERHOLSER AND R. H. TAYLOR

This work was undertaken as the result of an article by SHAMEL (8), in which he stated that a box of hard ripe Bartlett pears were placed in a lemon storage room where the temperature ranged from 79 to 100° F., with an average of 83.5°, the relative humidity varying from 85 to 96 per cent, with an average relative humidity of 85.1 per cent. The pears were subjected to these conditions from August 4 to September 3, 1916. Even though surrounded by these comparatively high temperatures, the pears remained hard and green until the end of the experiment (a period of 30 days). Within 6 or 7 days after being removed the pears ripened normally and were excellent to eat. As a check, SHAMEL compared these pears with other lots which had been stored in a room of a dwelling, where no attempt was made to control the temperature or relative humidity, but where one would assume both these factors would be lower than in the lemon house. Pears from this family storage room were ripe within a week, by August 10.

SHAMEL states that the "condition of high relative humidity was a controlling factor in retarding the ripening of the pears." He further states that "it is almost unbelievable that pears can be held for 30 days at the high temperatures recorded, without ripening or deteriorating." SHAMEL's observations seem startling when considered wholly from the viewpoint of experience in the

employment of cold storage and the utilization of low temperatures for the purpose of delaying the ripening of fruit. On the other hand, they seem to be in accord with certain observations which indicate that high temperatures, as well as low, may tend to retard the ripening process of fruit. In this connection the following observations of the writers are of interest.

When certain varieties of plums and cherries, early in their development upon the trees, are inclosed in closely woven, black sateen cloth sacks, there is a delay of 4 or 5 days in the attainment of maturity, and a prolonging of the period of edibility from 5 to 8 weeks after the crop of exposed fruits is normally harvested and eaten (7). At the time these data were presented, it was believed that light exclusion was the responsible factor; but in view of SHAMEL'S observations, it might have been high temperatures and high relative humidity in the area surrounding the fruits as a result of the covering of black sacks, the black cloth absorbing the heat rays and lessening the loss of moisture from the fruit. At least it is possible that the activity of the enzymes bringing about ripening was checked or partially inhibited.

BIOLETTI (3) has noted that European varieties of *Vitis vinifera* L. do not ripen in parts of California precisely according to the theory of ANGOT (1), who states that the buds of the European grapevine commence activity when the mean daily temperature reaches 9° C. From this point until the ripening of the grapes, the sum of the mean daily temperatures above 9° C. must reach 1130° C. for the earliest varieties, and 1520° C. for the latest. BIOLETTI finds that under Californian conditions the actual dates of ripening are from 2 to 4 weeks later than the time estimated by ANGOT, and that the greater delays in ripening are in the hotter localities. For example, in the Coachella Valley the seasonal sum of temperature above 9° C. from February to November is 5728° F. Accordingly, the grapes should ripen there from May 3 to May 23. As a matter of fact, the earliest varieties ripen about May 15–30, and the latest about June 15–30. BIOLETTI thinks that in these hottest regions the temperature of maximum acceleration may be passed, and intimates that the temperatures may become so high that a retarding effect upon the ripening is exerted.

Pears in the Vaca Valley, near Vacaville, California, have behaved in a way to indicate that high temperature may retard ripening. Although the Vaca Valley is famous for its early fruits, especially cherries and apricots, it is a well established fact that Bartlett pears grown there are notably longer in reaching maturity than those from any other section of northern California, unless it be from the mountain sections where the seasons are very late in opening, owing to their high elevation. One of the writers[1] has often seen a full crop of immature Bartlett pears still hanging on the trees in this valley when practically the entire crop was gone from orchards in both coast and interior valley sections. In the spring the pear trees blossom comparatively early, as do the other fruits. The young pears develop normally until the hot summer weather predominates, when they apparently almost cease growth, or at least grow slowly until cooler fall weather comes. Then the pears seem to commence growth again, often increasing noticeably in size and ripening in the normal way. It should be noted, however, that while the summer temperatures in Vaca Valley are generally unusually high, the relative humidity is practically always comparatively low.

In discussing SHAMEL'S interesting results and the results obtained by the writers, later recorded in this paper, WHITTEN, of the Division of Pomology, University of California, recalls observations which apparently bear upon this subject. He comments as follows:

> During the summer of 1901 there prevailed in the Mississippi valley the most severe drought and the highest temperatures recorded for that section since the United States Weather Bureau was established. During that season pears remained firm on the trees much later than in normal years. In numerous instances varieties were exhibited at fall and winter fruit shows in Missouri, weeks later than the same varieties ordinarily keep for exhibition. Similar retardation, but to a less degree, of the development of pears, in the same section, has been observed to occur during occasional subsequent dry, hot summers.
>
> The casual explanation, usually offered at that time, was that the development of the pears was retarded by unfavorable conditions for growth, and that

[1] Observations made in Vaca Valley during the growing seasons since 1912 by TAYLOR.

this retarded development resulted in later ripening. The results of investigations initiated by SHAMEL seem to justify the further interpretation that tardy ripening during unusually hot summers may have been due to the high temperatures opposing the ripening process.

Clusters of grapes, included in sacks during their growing period, ripen later and keep decidedly longer than do similar grapes not protected by sacks. Their longer keeping has been regarded as being due to protection afforded by the sacks from injurious agencies. It is now possible to assume, however, that the higher temperature within the sack may account, in part at least, for both later ripening and longer keeping qualities.

CARDINAL TEMPERATURES.—As is well known, certain cardinal or fundamental temperatures are recognized. "Maximum" and

TABLE I

PLANT	MINIMUM	OPTIMUM	MAXIMUM
	Cardinal temperatures for growth, ° C.		
Corn	4.8–10.5	37–44	44–50
Pea	0.0– 4.8	25–31	31–37
Cucumber	15.6–18.5	31–37	44–50
Wheat	0.0– 4.8	25–31	31–37
Barley	0.0– 4.8	25–31	31–37
	Cardinal temperatures for germination, ° C.		
Corn	9.4	34.0	46.2
Pea	9.4	34.0	46.2
Cucumber	14.0	34.0	46.2
Wheat	5.0	29.0	42.0
Barley	5.0	29.0	37.5

"minimum" are terms used to refer to the highest and lowest temperatures at which the development of a particular organism may occur. The most favorable temperature for any process or function is designated the "optimum." The optimum temperatures as a rule do not have a wide range. A variation of 5 or 6° one way or the other may be sufficient to have an appreciable effect upon the process or function involved. Furthermore, it is known that there may be separate maxima and minima for every process or activity or tissue of the plant. As shown in table I, HABERLANDT (6) gives a comparison of the relation of the different activities of a few plants to these cardinal temperatures. These figures are only suggestive, because the particular variety of the

same species and the other environmental factors would affect the cardinal temperatures.

It would not necessarily follow that the best temperature for the greatest vegetative growth of pears, for example, would likewise be the most favorable for fruit development, and this is generally recognized by growers. Furthermore, the most favorable temperature for the growth of the fruit on the tree may not be the optimum for continued ripening of the fruit after harvesting, with best flavor and resulting texture.

INHIBITION AT HIGH TEMPERATURES.—The fact is well known that metabolism, enzyme action, and other processes or functions of the plant are to a certain point rapidly increased with a rise in temperature. BLACKMAN (4), however, has shown that the maximum activity, especially for respiration and photosynthesis, has commonly been placed too high, since proper consideration of the time factor has not always been given. Above a certain point it has also been clearly shown that high temperatures weaken and lessen general metabolic activities.

From work done by BALLS (2) it is possible that the inhibition of growth at high temperatures during a considerable period of time may be the result of an accumulation in the cells of injurious metabolic products. BALLS thinks that some of these deleterious products are produced at low temperatures, but under such conditions they are decomposed about as rapidly as formed. At high temperatures, however, production is more rapid than decomposition, and accumulation takes place which results in the injury or inhibition of metabolism.

GORE (5), using temperatures from 2° to 35.6° C., found the rate of respiration increased an average of 2.376 times for each 10° C. rise in temperature for 49 sets of determinations, with 40 different kinds of fruits. An interesting statement by GORE is that "with many fruits the activity has been found to decline when held at high temperatures."

Experiment 1

In view of SHAMEL'S report and the degree to which it seemed to be substantiated by minor similar experiments and observations

of the writers, it was decided to conduct the following preliminary experiments. While SHAMEL believed it was the high relative humidity which was the controlling factor in retarding the ripening of the pears, nevertheless the factor of high temperatures was also present. Hence an experiment was outlined to endeavor to determine whether high temperatures, or humidity, or both were responsible.

METHOD

To obtain for the test what appeared to be the more important combinations of temperature and humidity, compartments were arranged as follows: (1) To maintain high temperature and high humidity a large drying oven, having a ventilation outlet at the top, was arranged with four shelves above two electric heaters. Between the heaters and the shelves were buckets of water with sacks and towels hanging into them to increase the evaporating surface. (2) For high temperature and low humidity a Freas electric oven was used with sufficient ventilation to maintain a comparatively low relative humidity, but sufficient heat to maintain a comparatively high temperature. (3) Two lockers were maintained at room temperature, one with ordinary humidity of the room and the other with provision for maintaining a high relative humidity. (4) The cold storage room where a check lot of pears was kept, was maintained constant by means of a thermostat, so that the temperature was always between 30.5° F. and 32.8° F., with the relative humidity ranging from 67 and 73 per cent. Throughout the experiment, which continued for 21 days, one hygrothermograph was kept on the third shelf (next to the bottom shelf) in the large drying oven, and another in the locker with normal temperature and high humidity. These were both checked several times by wet and dry bulb psychrometer and tested mercurial thermometers.

Eight 5 lb. grape baskets were filled with Bartlett pears and placed at noon on September 2 in the various situations. Each lot was numbered and described as follows:

Lot 1, top shelf (no. 1) of large oven; high temperature 85° F. and high humidity 100 per cent.

Lot 2, next to top shelf (no. 2) of large oven; high temperature 88° F. and high humidity 100 per cent.
Lot 3, next to bottom shelf (no. 3) of large oven; high temperature 94° F. and high humidity 91 per cent.
Lot 4, bottom shelf (no. 4) of large oven; high temperature 104° F. and moderate humidity about 60 per cent.
Lot 5, in small Freas electric oven; high temperature 95° F. and low humidity well below 50 per cent.
Lot 6, ordinary locker in concrete building; room temperature 71° F. and room humidity about 60 per cent.
Lot 7, ordinary locker in concrete building; room temperature 69° F. and high humidity 92 per cent.
Lot 8, held in cold storage at between 30.5° and 32.8° F. and a humidity ranging from 67 to 73 per cent.

Observations on temperature and humidity

In addition to the continous hygrothermograph records made by lots 3 and 7, the writers made careful check readings on thermometers at intervals of 1 to 4 days apart. For reference, these are given in table II.

TABLE II

Temperature records during storage tests

Date	Time	Temperature of lots in °F.							
		1	2	3	4	5	6	7	8†
September 2...	11:10 A.M.	85.5	89.5	94.0	106.0	.....	.....	.....	31.3
3...	3:00 P.M.	84.0	89.0	92.0	104.0	86.0	70.7	70.0	31.0
6...	9:45 A.M.	83.2	89.0	95.2	107.6	87.0	69.2	68.0	32.8
7...	9:00 A.M.	84.5	90.0	95.7	107.0	.....	.....	68.5	31.4
9...	12:15 P.M.	88.5	90.0	98.0	112.1	.....	69.2	67.5	30.5
10...	3:45 P.M.	84.5	90.0	95.7	107.6	96.8	70.0	68.0	32.7
14...	12:00 Noon	84.0	87.0	92.2	100.8	97.7	72.7	70.2	31.4
16...	12:15 P.M.	.....	85.5	93.0	102.2	96.8	71.0	69.0	31.7
19...	1:45 P.M.	.....	86.0	93.2	103.1	93.6	72.0	69.5	32.4
20...	11:45 A.M.	.....	.....	84.0	86.5	94.1	.....	.....	32.8
20...	5:40 P.M.*	.....	.....	77.0	77.0	.....	.....	.....	.....
21...	11:45 A.M.	.....	.....	92.0	96.1	101.3	.....	.....	30.8
23...	9:45 A.M.	.....	.....	100.5	107.2	103.1	.....	.....	30.7
Average..	.....	84.9	87.7	93.9	103.7	95.0	70.7	68.9	31.7
Maximum.	.....	88.5	90.0	100.5	112.1	103.1	72.7	70.2	32.8
Minimum.	.....	83.2	85.5	77.0	77.0	86.0	69.2	67.5	30.5

* Electric current off from 11:45 A.M. to 5:40 P.M. only.

† Temperature with lot 8 in cold storage remained quite uniform, rising to the maximum and dropping to the minimum with each run of the compressor about every 3 hours.

The records were made immediately on first opening the doors to the ovens or other compartments, two observers working together. During the time observations were being made, the temperatures as well as the humidity dropped, but the hygrothermograph charts show that under the high temperatures prevailing in the large oven, normal conditions were restored in 30 minutes to 2 hours as regards temperature, and in 1 to 2 hours as regards humidity. In the locker with lot 7, with air temperature normal,

TABLE III

HUMIDITY RECORDS DURING STORAGE TESTS

Date	Time	Percentage of relative humidity of lots				
		1	2	3	7	8*
September 2...	11:10 A.M.	100	100	92.0	.....	69.0
3...	3:00 P.M.	100	100	.....	82.0	68.0
6...	9:45 A.M.	100	100	89.0	84.0	73.0
7...	9:00 A.M.	100	100	88.0	92.0	69.0
9...	12:15 P.M.	100	100	82.5	96.0	67.0
10...	3:45 P.M.	100	100	83.0	91.0	73.0
14...	12:00 Noon	100	100	94.0	96.0	69.0
16...	12:15 P.M.	.....	100	93.0	98.0	70.0
19...	1:45 P.M.	.....	100	89.0	97.0	72.0
20...	11:45 A.M.	.....	.....	100.0	.....	73.0
21...	11:45 A.M.	.....	.....	98.0	.....	68.0
23...	9:45 A.M.	.....	.....	93.0	.....	68.0
Average....	.....	100	100	90.7	91.7	70.0
Maximum..	.....	100	100	100.0	98.0	73.0
Minimum...	.....	100	100	82.5	82.0	67.0

* Humidity with lot 8 in cold storage remained quite uniform, rising to the maximum and dropping to the minimum with each run of the compressor about every 3 hours.

high humidity was restored in 4 to 10 hours after closing the door. In no case, however, did the humidity drop below 90 per cent and remain there for more than one hour. The slow rise from 95 to 100 per cent, or to saturation, required the longest time.

The observations on humidity are shown in table III. Lot 4 ranged about 60 per cent humidity; lot 5 ranged well below 50 per cent; and lot 6 ranged from 53 to 65 per cent humidity. Lots 1 and 2 are indicated as having always been in a saturated atmosphere. This was assumed from the fact that every time the door was opened to take readings, the walls, top, and bottom of

the shelves were covered with drops of precipitated moisture, and the wrapping paper surrounding the fruits was always moist. This was not generally true with lots 3 and 4. The condition of the fruit itself, as indicated by its wilting, should serve as a good indication of the relative humidity of the atmosphere surrounding the various lots. This will appear later.

Behavior of fruit

In the beginning of the experiment all the pears were very similar in degree of ripeness, all being yellowish green and about one-fourth ripe, as indicated by color. Degree of ripeness may be described from two standpoints, namely, appearance, indicated largely by color, and condition, indicated by texture, juiciness, and flavor. It was possible to describe the former as a certain fraction ripe, and the fractions in table IV refer to ripeness in appearance only, unless otherwise noted. Additional statements cover condition. The pears in each lot were examined at approximately 4-day intervals, and careful notes made as to appearance and condition. The somewhat abridged notes in table IV indicate the condition of the fruit as the experiment progressed.

The experiment was continued beyond September 23, but on the 25th an accident in the regulation caused the temperatures to climb abnormally high in the box where nos. 1–4 were located. The result was that the pears in lots 3 and 4 were cooked brown, so that further observations were impossible. It was interesting to note, however, that lot 3 was cooked much more severely than lot 4. The temperature of lot 3 as compared with lot 4 was approximately 10° lower, while the relative humidity was about 30 per cent higher. Just before this, one fruit each from lots 3 and 4 were placed where lot 7 had been at room temperature and high humidity, to discover whether these fruits would ripen normally after removal from the high temperature. These fruits were observed and sampled on September 28. No. 3, although noticeably wilted on September 23, had by the 28th become apparently more plump, appearing almost normal. The fruit was full soft ripe; flesh rather tough; and flavor more acid than normal, with a faint trace of bitterness, although this may have been due

TABLE IV

CONDITION OF PEARS DURING PROGRESS OF STORAGE TEST

LOT NO.	TREATMENT	DATE OF EXAMINATION				
		September 6	September 10	September 14	September 19	September 23
1.....	High temperature (85° F.), high humidity (100 per cent)	Three-fourths ripe, greenish yellow to yellow, all in good condition, plump	Full ripe, clear yellow, medium firm ripe condition, few small breakdown spots on surface	Past ripe, soft, considerable breakdown and decay developing*	.....	.....
2.....	High temperature (88° F.), high humidity (100 per cent)	Two-thirds ripe, greenish yellow, all in good condition, plump	Four-fifths ripe, plump, firm, unripe	Nine-tenths ripe, full yellow, firm ripe, few fruits showing small decay spots	60 per cent breakdown and mold, rotten*	.....
3.....	High temperature (94° F.), high humidity (91 per cent)	One-half ripe, yellowish green to greenish yellow, all in good condition, plump	Two-thirds ripe, greenish yellow, hard, unripe, faint trace of wilting	Three-fourths ripe in color and texture, some fruits plump, others very slightly wilted	Nine-tenths ripe in appearance, nearly to full yellow, four-fifths ripe in texture, hard or very firm ripe, slightly wilted	Fully colored yellow, firm, unripe, wilted
4.....	High temperature (104° F.), moderate humidity (estimated 60 per cent)	One-third ripe, yellowish green, one pear rotten, others good, plump	One-half ripe, yellowish green to greenish yellow, perceptibly wilted, one with complete breakdown in lower one-third of length	One-half ripe, practically the same as on September 10	Two-thirds ripe, greenish yellow, two-thirds ripe in texture	Three-fourths to four-fifths colored yellow, hard unripe, rather badly shriveled, dry

5.....	High temperature (95° F.), low humidity (less than 50 per cent)	Five-sixths ripe, yellow, wilting slightly	Thermostat out of adjustment, temperature too high, fruit cooked; new lot from storage to replace old	One-half ripe (almost), nearly as ripe as lot 4	Two-thirds ripe, same as lot 4	Three-fourths to four-fifths colored yellow, same as lot 4 in every way
6.....	Room temperature (71° F.), room humidity (60 per cent)	Nine-tenths ripe, nearly full yellow, firm ripe, plump	Almost as ripe as lot 1	Full ripe to past ripe, or medium to soft ripe, undisturbed fruits in best condition	Dead ripe to past, all fruits show more or less breakdown*	.....
7.....	Room temperature (69° F.), high humidity (92 per cent)	Five-sixths ripe, yellow, plump, good condition	Practically as ripe as lot 1	Full ripe to slightly past, no noticeable difference as compared with lot 6	Same as lot 6*	.....
8.....	Cold storage (32° F.), moderate humidity (70 per cent)	One-fourth ripe or less	Remained practically the same throughout period of experiment.			

* Removed from the experiment.

to the absorption of the odor from the cedar wood closets in which the fruit was held. At any rate, the ripe fruit was much poorer in quality than the Bartlett at its best when ripened at normal temperatures. No. 4 was still as wilted as before. Fruit was full ripe, but dry and tough. This fruit remained about as wilted as when first placed. The fruit was not soft, but as much so as it ever would be without being well past ripe. It was very inferior in flavor and quality, much the same as no. 3. Lot 8, which was held in cold storage throughout the progress of the experiment, showed almost no appreciable ripening, being practically as hard and unripe at the end of the month as at the beginning.

Discussion of results

The pears in lot 1, placed at a temperature averaging about 85° F. and in a saturated humidity, were full ripe 8 days after being subjected to the conditions. A study of table IV shows that the pears in lot 2, placed at a temperature averaging 87.7° F. and in a saturated atmosphere, were full ripe about 13 days after being subjected to the conditions. Since the fruit was all in the same stage of maturity before the experiment started, this would show a delay of 5 days in ripening, which can only be accounted for by the fact that the temperature was about 3° higher.

The pears in lots 6 and 7 were also full ripe 8 days after the experiment started. The temperature surrounding lots 6 and 7 was practically the same in both cases, and averaged about 70° F. The difference in the conditions surrounding these two lots was in the humidity. The humidity in the compartment containing lot 6 was fairly constant, about 60 per cent; the humidity surrounding lot 7 averaged about 92 per cent. The temperatures alike, the difference in humidity showed no effect upon the ripening. Furthermore, when compared with lot 1, the fruit ripened with approximately the same rapidity at temperatures of 70 and 85° F. The pears in lot 3 remained firm unripe for 3 weeks after being subjected to a temperature averaging about 94° F. and a humidity of 91 per cent. This shows a delay of 13 days when compared with lots 1, 6, and 7. This apparently was due to the somewhat

greater temperature at which the pears were kept. The somewhat lower humidity resulted in the pears wilting appreciably. The pears in lot 4 were hard unripe, or not quite as ripe as the fruit in lot 3. The temperature averaged about 104°F. and the humidity approximately 60 per cent. The higher temperature resulted in an appreciable delay in ripening when contrasted with lot 3, but the lower relative humidity caused considerable wilting. With the high temperatures some difficulty was experienced in maintaining as high humidity as was desired in the case of lots 3 and 4.

Interpretation of results

It is somewhat difficult to account for the surprising results obtained. The general idea has been that low temperatures only were of importance in preserving fruits for any period of time and in arresting the deteriorating processes. As contrasted with this, high temperatures were looked upon as extremely conducive to a hastening of the breakdown of the tissues and in shortening the keeping period of fruit.

The delay in ripening might be assumed upon the basis of an accumulation of carbon dioxide, the assumption being that possibly a comparatively large mass of fruit stored in a relatively small closed container, at high temperatures, would result in an abnormal amount of carbon dioxide surrounding the fruit. The writers, however, doubt whether there was any measurable accumulation of carbon dioxide, since the capacity of the drying oven was relatively large for the amount of fruit contained therein. Furthermore, the ventilation pipe at the top permitted the warm air to be continually escaping. In addition, the oven was opened about every 3 days to make observations and add water to the evaporating pan. This would give a good aeration. The writers at first felt that the explanation might be that with certain low temperatures conditions result whereby not only katabolic activity or destructive metabolism but all metabolism is lessened or reduced to a minimum. On the other hand, with high temperatures and high relative humidity surrounding the fruit, conditions may be produced whereby the tissues are able, at least partially, to carry on anabolic activity or constructive metabolism, and hence

indirectly lessen the amount of rapidity of activity which would bring about deterioration.

As a result of further work, however, it seems probable that within a given limit high temperatures may act in the same manner as do the low temperatures to which fruits are subjected in cold storage; that is, temperatures approaching certain limits in either extreme cause a reduction in the protoplasmic and enzymatic activities of the fruit, and this, depending upon the extent of the inhibition, delays to a greater or less degree the attainment of ripeness. As has been stated elsewhere, the experiments reported upon are of a very preliminary nature, and an effort is being made to repeat them. Furthermore, at such high temperatures for any long period of time the flavor might be affected so that the quality would be appreciably lowered. As a matter of fact, the flavor of the pears subjected to the higher temperatures was somewhat abnormal. A slight acidity was noticeable and a lack of the normal sweetish taste and juiciness was apparent. This can probably be accounted for by the fact that the comparatively high temperatures would be expected to increase the respiration. Carbohydrates are necessary for respiration, and are gradually used by this process; hence it follows that the sugar content would have been decreased. This decreasing of the sugar content would have made the normal acid content somewhat more noticeable, and, in addition, it is possible that intramolecular respiration may have been carried on to a certain extent, and this give rise to waste products that affect the flavor.

A second drawback to the practicality of utilizing high temperatures and high humidity in keeping fruits is the danger from rot. Under such an environment, conditions are very favorable for the growth of fungi or bacterial organisms which would bring about the decay of the fruit. While the experiments, therefore, show that temperatures ranging from 95 to 110° F., with the optimum at about 104 or 105° F., will delay or prolong the normal ripening process of Bartlett pears at least two weeks, when contrasted with fruit placed at average room temperatures of 70 to 80° F., the danger from rot and the development of abnormal flavors limit the practical use of these higher temperatures.

Experiment 2

In the preceding experiment with the highest temperature used (104° F.) the Bartlett pears kept longest. The authors wished to ascertain whether temperatures higher than those employed in the first experiment would be more satisfactory. To determine this, and also to repeat in a measure the first experiment, a second experiment was conducted.

Observations on temperature and humidity

The method of procedure was just as outlined for the first experiment, except that the temperatures in the large drying oven were somewhat higher than was the case in experiment 1; that is, the top shelf (no. 1) had a temperature averaging 90° F. as contrasted with 85° F. in the first experiment; shelf no. 2 averaged 99.2° F. instead of 88° F.; shelf no. 3 averaged 109° F. instead of 94° F.; and shelf no. 4 averaged 121.2° F. as compared with 104° F. The Freas oven averaged about 101° F.; while in the first experiment it was kept at about 95° F. The other temperatures and the humidity were just about the same as for the first experiment.

The experiment was begun on September 25, 1918. One set consisted of 5 lb. grape baskets filled with first crop Bartlett pears; a second set consisted of second crop Bartletts; and the third set was filled with Easter pears. One lot of each set was placed under each of the varying conditions. By an improved arrangement for maintaining a high humidity, it was possible to fill the water pan from outside without opening the door of the oven. Since the writers knew just about what to expect from the large oven as well as the other compartments, and since the hygrothermographs were operated throughout this experiment as in the first one, it was not found necessary to open the door at frequent intervals to take readings. The hygrothermograph showed that the temperatures and humidity were quite uniform, in fact more so than in the first experiment because of better control, except on two occasions. The first was from noon, September 28, to noon, September 30. During this time the water pan was dry and the humidity dropped considerably below 50 per cent. At the same

time the temperature rose from 4 to 6° F. only above the temperatures indicated, as shown by the continuous record of the thermograph pen. The second was during the last 36 hours of the experiment, ending October 10, when the pan again went dry. The operation of the thermostat prevented any rise in temperature above the normal. In fact the thermostat was so closely adjusted that the variation in temperature at the hygrothermograph was only from 1 to 2° F. at any time except when the door was open.

The variation in humidity was somewhat greater on the bottom or fourth shelf, although it was probably close to 90±5 per cent. On the first three shelves the humidity was 100 per cent throughout the experiment, except during the times indicated. Room temperatures and humidity were practically the same as in the first experiment, not more than 1° F., or 6 per cent difference between the maxima and minima. The high humidity at room temperature was quite uniform, ranging from 94 to 100 per cent, average 96 per cent. The cold storage temperature and humidity were just the same as in the first experiment.

Behavior of fruit

The first crop Bartlett pears were near the end of their life period when first subjected to the experimental conditions. As a result, 3 or 4 days after the experiment was begun nearly all the specimens were physiologically broken down, as indicated by the blackening of the skin, and the browning and extreme softening of the tissue. No data of value, therefore, concerning the effects of high temperatures upon keeping quality, were obtained with this lot of nearly ripe Bartlett pears.

The second crop Bartlett and the Easter pears were green enough to show a response, with wide enough differences, depending upon the temperature, to be of interest in substantiating the first experiment, and to determine the effects of temperatures higher than those employed in the first test. The details of these are given in tables V and VI.

As indicated by the nearly ripe Bartlett pears, there is a point near the stage of complete maturity in ripening at which breakdown may rapidly come about, regardless of the environment.

TABLE V

CONDITION OF SECOND CROP BARTLETT PEARS DURING PROGRESS OF SECOND TEST, SEPTEMBER 25 TO OCTOBER 10, 1918

LOT NO.	TREATMENT	DATE OF EXAMINATION		
		September 28	October 3	October 10
1.....	High temperature (90° F.), high humidity (100 per cent)	Only slightly riper than when started	Firm, unripe, greenish yellow, extreme saturation developing mold on fruits	Firm, no wilting, nearly yellow, three-fourths colored, not yet ripe
2.....	High temperature (99.2° F.), high humidity (100 per cent)	Only slightly riper than when started	Firm, unripe, supersaturated atmosphere developing mold	Firm, no wilting, nearly yellow, not quite as ripe as lot 1
3.....	High temperature (109° F.), high humidity (91 per cent)	Only slightly riper than when started	Firm, unripe, greenish yellow; 1 fruit slight browning at stem end, others small brown rotten spots	Firm, no wilting, nearly yellow, not quite as ripe as lot 2; 3 fruits slight breakdown at base, lacking in flavor, quality very poor
4.....	High temperature (121.2° F.), moderate humidity (70 per cent)	Slight browning of skin to one-third breakdown	All chocolate colored throughout, cooked taste, quite firm	All gone
5.....	High temperature (101° F.), low humidity (well below 50 per cent)	Same as lot 3	Firm, unripe, greenish yellow, same as lot 3	Firm, wilted, not ripe, same as lot 1*
6.....	Room temperature (71° F.), room humidity (60 per cent)	About the same as lot 1	Full ripe, almost at best eating condition, light yellow, slightly wilted	Soft ripe, full clear yellow, at or slightly past best eating, no wilting
7.....	Room temperature (69° F.), high humidity (96 per cent)	Same as lot 6	Same as lot 6	Same as lot 6
8.....	Cold storage (32° F.), moderate humidity (70 per cent)	Same as when test started	Same as when test started	Practically same as when test started

*The second crop Bartlett pears of this lot was allowed to remain in the Freas oven until November 5 On this date, nearly 6 weeks after the experiment was started, the fruit could be described as five-sixths colored, unripe, dry, rather badly shriveled, and somewhat insipid, but not displeasing in flavor.

TABLE VI

CONDITION OF EASTER PEARS DURING THE PROGRESS OF THE SECOND TEST

LOT NO.	TREATMENT	DATE OF EXAMINATION		
		September 28	October 3	October 10
1.....	High temperature (90° F.), high humidity (100 per cent)	Green, same as when first put in, some mold developing	Green, firm, unripe; half of fruits largely or completely rotted with *Rhizopus* and *Penicillium* mold	Firm, unripe, lighter green than when put in
2.....	High temperature (99.2° F.), high humidity (100 per cent)	Green, same as when first put in, some mold	Green, firm, unripe; half of fruits largely or completely rotted with *Rhizopus* and *Penicillium* mold	Firm, unripe, greenish yellow; considerable mold
3.....	High temperature (109° F.), high humidity (91 per cent)	Green, same as when first put in, no mold	Green, firm, unripe; only slight mold at base of 2 fruits	Completely broken down, and rotted with mold, skin chocolate brown, flesh dirty white color
4.....	High temperature (121.2° F.), moderate humidity (70 per cent)	Green, same as when first put in, no mold	All fruits seemed cooked; skin chocolate colored with tissue soft and grayish white with flecks of brown scattered through, considerable internal breakdown	.....
5.....	High temperature (101° F.), low humidity (well below 50 per cent)	Green, same as when first put in, no mold	Same as lot 3, noticeably wilted	Unripe, light green with few small patches of yellowish green, very badly wilted

TABLE VI—*Continued*

Lot no.	Treatment	Date of examination		
		September 28	October 3	October 10
6.....	Room temperature (71° F.), room humidity (60 per cent)	Green, same as when first put in, no mold	Green, firm, unripe, very slightly wilted	Medium firm ripe, yellowish green, almost at best eating, somewhat wilted
7.....	Room temperature (69° F.), high humidity (96 per cent)	Green, same as when first put in, no mold	Same as lot 6, but even less wilted, practically plump	Firm ripe, light yellowish green, almost as ripe as lot 6
8.....	Cold storage (32° F.), moderate humidity (70 per cent)	Green, same as when first put in, no mold	Same as when put in	Same as when put in

This is true notwithstanding the fact that earlier in the period of ripening certain identical conditions, as contrasted with others, would appreciably arrest the ripening process.

The results of experiment 2 indicate that for Bartlett pears a nearly continuous temperature of 104–110° F., and a relative humidity of 95–98 per cent, result in the most marked delay of the ripening process when high temperatures are the factor employed. Temperatures above 110° F. result in a more rapid breakdown of the tissue than do any temperatures below. A temperature of 107° F. gives better results in delaying the ripening than 110° F. When the moisture content of the surrounding air is so high that water is precipitated on the fruit, the pears do not keep nearly as well as when the relative humidity is just sufficiently low to prevent this. This second experiment shows rather conclusively that within a certain limit high temperatures tend appreciably to delay the ripening of Bartlett and Easter pears. Excessively high humidity and these high temperatures, however, make conditions favorable for the infection and growth of fungi upon the pears. Low humidity and these high temperatures, of course, result in excessive wilting of the fruit.

Table V shows that the second crop Bartlett pears designated as lot 5 were of especial interest, in that they remained unripe for a relatively longer period than any of the other lots. Lot 5 was in the Freas oven at a temperature of 101° F. and surrounded by a relative humidity below 50 per cent until November 5. On this date, nearly 6 weeks after the beginning of the experiment, the pears were still unripe. When compared with fruits stored at room temperatures, this shows a delay in ripening of a little over 4 weeks. This lot also is of interest in that it indicates that it is a question of high temperature only, which causes the ripening processes to be inhibited, and that high relative humidity has no marked influence, except to lessen the amount of wilting.

The question arises why the fruit of lot 5 should keep longer than the fruit of lots 2 and 3, since the temperatures in each case were all comparatively high. The chief difference between these lots was the much lower relative humidity of lot 5, as contrasted with lots 2 and 3. It is probable that the greater desiccation or wilting of the pears of lot 5 did retard their ripening, but two other points should be mentioned. (1) When the relative humidity was high, much trouble was experienced from molds infecting the fruit and causing it to rot. There was no loss from rot in lot 5, due no doubt to the very low humidity. (2) The temperature of lot 3 was no doubt too high, and it is probable that the temperature surrounding lot 2 was somewhat below the optimum temperature for the retardation of the ripening.

Specimens from lot 5 were tested by Dr. J. RUDISCH and the senior author to determine if any enzymes were active. The tissue was treated with a tincture of guiac and gave no test for oxidase, either with or without the addition of hydrogen peroxide; neither could a test for an organic peroxide be shown upon the addition of a solution of potassium iodide, weak acid, and starch solution, as indicated by the liberation of free iodine and the consequent blueing of the starch solution. This might indicate that the higher temperatures had destroyed or inhibited the action of the ferments or enzymes normally present in the tissue of pears. This resulted in a checking of the ripening process with a consequent prolonging of the period in which the fruit could be kept.

Experiment 3

Effects of high temperatures upon keeping apples

Since Bartlett and Easter pears behaved in such an unexpected manner when subjected to temperatures of around 104° F., an endeavor was made to determine whether varieties of apples would behave in a similar manner. Yellow Newtown apples, which had previously been kept in cold storage at a temperature of 32° F., were subjected to high temperatures similar to the process in experiment 2.

The experiment was begun on December 12, with a 5 lb. grape basket filled with apples subjected to each of the several conditions. The temperatures varied as follows: 32, 70, 85, 95, 104, 110, and 120° F. The relative humidity was from 90 to 98 per cent in each case, except that the temperatures of 70° F. and 104° F. were duplicated, the relative humidity in one instance being somewhat below 50 per cent and in the other varying from 90 to 98 per cent. The results of this experiment can be summarized briefly. The ripening of the apples was not delayed by the higher temperatures. The rapidity of ripening was directly proportional to the temperature, in that with the degrees tried the higher the temperature the more rapid the ripening. After 2 weeks the fruit subjected to temperatures of 85° F. and above were all browned throughout and soft, tasting very much like baked apples. The fruit at 70° F., or room temperature, was yellow in color, ripe, and just about best for eating. The fruit at 32° F. was still green and hard unripe.

Practical applications

The practical applications of the data presented are somewhat limited, but the facts may be of value some years and in certain sections in connection with the time of picking Bartlett pears. For example, as a rule during the hottest seasons the growers have felt a greater necessity for earlier picking than when the season is normal at the time of ripening. In view of the results obtained, it may really happen that the ripening of the pears is delayed by the excessively hot weather, and would mean that the fruit might well be allowed to remain on the trees longer than would be the

case in a normal season. This would be of especial value when fruit was being harvested and packed for eastern shipment. Pears are picked comparatively early in order to reach distant markets in good condition. While they should preferably not be allowed to ripen on the tree, to avoid the marked development of the grit cells, it might mean that in excessively hot years, contrary to expectations, the fruit could be left somewhat longer on the trees, and thereby develop a better flavor and quality. If all varieties of apples behave as do Yellow Newtown, high temperatures do not delay ripening. Instead, up to the point of tissue destruction by heat, the higher the temperature, the more rapid the ripening. This emphasizes the necessity of hurrying into low temperatures apples which are to be stored for any length of time.

Summary

1. When contrasted with temperatures between 70 and 85° F., temperatures of 87.7 to 110° F. caused an appreciable delay in the ripening of green first crop Bartlett pears.

2. The retardation of ripening was directly proportional to the increased degree of heat within the limits of 87 and 104° F.

3. The amount of delay in ripening of green first crop Bartlett pears of the different temperatures when contrasted with 70° F., or room temperature, was as follows: 85° F., no retardation; 87.7°, 5 days; 94° F. and 104° F., 13 days.

4. Second crop Bartlett pears, placed at a temperature of 101° F. and surrounded by a relative humidity of below 50 per cent, remained unripe 4 weeks after similar pears had become fully ripe at room temperature and humidity.

5. The relative humidity does not seem to be a significant factor in checking the ripening processes. Its effect is in lessening or permitting wilting, depending upon whether the relative humidity surrounding the fruit is high or low.

6. The flavor of the pears subjected to those temperatures higher than 85° F. was not normal. There was a slight acidity, and the sweetish taste and juiciness were lacking.

7. Temperatures above 110° F. result in a more rapid ripening and consequent breakdown of the tissue than do any of the lower temperatures, down to average room temperatures.

8. As would be expected, there was a comparatively large loss from rot with the fruit kept at high temperatures and surrounded by high relative humidity.

9. A possible explanation of the effects of high temperatures may lie in the influence upon the enzymes. Temperatures approaching the probable minimum (around 28° F.) on the one hand, and the probable maximum (around 110° F.) on the other, might result in a reduction of enzymatic activities of the fruit and a consequent retardation of the ripening processes; while with the optimum temperatures (70–85° F.) the enzymatic activity would be most marked, and hence the ripening most rapid.

10. If the Bartlett pears have nearly reached a stage of complete ripeness, the temperatures above 70° F. do not check the ripening process. On the other hand, the ripening and breakdown are more rapid with each appreciable rise in temperature.

11. Unripe Easter pears behave in a manner comparable to the Bartlett when placed under similar conditions of high temperatures and relative humidity.

12. The process of ripening with Yellow Newtown apples is not delayed by temperatures above 32° F. The ripening takes place with increased rapidity with each appreciable rise in temperature above 32° F. This is true with temperatures up to a point which result in the disorganization of the protoplasmic contents of the cells.

13. The experiments suggest that with an excessively hot season during the time of ripening, Bartlett and Easter and possibly other pears might be allowed to remain on the trees somewhat longer than with a normal season.

14. For Yellow Newtown and no doubt other varieties of apples, which are to be stored any length of time, the necessity of quickly cooling after harvesting is emphasized.

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DIAPHRAGMS OF WATER PLANTS

II. EFFECT OF CERTAIN FACTORS UPON DEVELOPMENT OF AIR CHAMBERS AND DIAPHRAGMS

LAETITIA M. SNOW

(WITH THREE FIGURES)

The experiments reported in this paper were started at Wellesley College in 1914–1915, and were continued at the University of Chicago during the winter of 1915–1916. It was intended to repeat the experiments and confirm the results, but as it has been impossible to do so, it seems better to report the work in its present incomplete condition than to delay its publication any longer. Thanks are due the Association of Collegiate Alumnae for the grant of the Alice Freeman Palmer Memorial Fellowship for 1915–1916, the Missouri Botanical Garden for the material of *Scirpus validus* which was collected and started at St. Louis, and the botanical staff of the University of Chicago for their cordial co-operation in placing the facilities of the laboratory at my disposal.

Water

As the general impression is that an increase in the water content of the soil produces an increase in the amount of air-containing tissue, culms of *Scirpus validus* were allowed to grow alternately under water and in the air, in order to note the effect of the change upon the air spaces and diaphragms.

EXPERIMENT 1

In order to be sure that the part studied actually grew under the desired condition, it was necessary to ascertain the region of growth of the stem. Consequently in 1914–1915 culms were marked in 2 mm. sections from the tip downward. In some cases the marks extended to the sheathing scale leaves at the base (called "to sheath" in table I); in others the sheath was stripped off and the marks carried down the stem to the rhizome (called "culm" in the table). DD 5 showed a discrepancy between the

millimeters of growth and the distance of the last mark from the ground. As it grew 33 mm. and the last mark was 38 mm. from the ground, the difference of 5 mm. might mean that three marks had disappeared. If the first was at the base, the stretching, in destroying these marks, could not have extended farther than 5 mm., as the last mark visible was clear cut and 2 mm. from the one next above it. The discrepancy was more likely to have been the result of faulty measurements.

TABLE I

REGION OF GROWTH IN *Scirpus validus*

Pot	Culm no.	Marking	Region of growth
B	1	To sheath	No growth
B	2	To sheath	Below top of sheath
C	3	To sheath (?)	Below top of sheath
C	4	To sheath	No growth
F	3	To sheath (?)	No growth
F	4	To sheath	Below top of sheath
G	4	Culm	Below last mark
G	5	Culm	Below last mark
BB	4	To sheath	Below top of sheath
DD	2	To sheath	No growth
DD	4	Culm	Below last mark
DD	5	Culm	Below last mark (?)

EXPERIMENT 2

In February 1915, 11 culms were marked, 6 to the top of the sheath and 5 on the stem to the base. After a period of growth all marks were clear cut, and showed no separation. This may mean that all growth took place below the last mark, or that some of the marks had disappeared. To test this, experiment 3 was started.

EXPERIMENT 3

In March 1915, 4 culms were marked to the base, as just described, and the number of marks counted. One culm did not grow. In the second culm, after one day, the lowest mark had disappeared; the second mark was 4 mm. from the base and was perfectly clear cut. The other marks had not changed. In the third culm, after 7 days, the first mark was at the base on a piece of sheath; the second was clear and 13 mm. above the base. In the fourth culm, after 7 days, the first mark was on a piece

of sheath; the second was clear and 4 mm. from the base. The third culm was marked again, and by the next day the first mark had disappeared and the second mark was 4 mm. from the base. From these experiments it seems reasonable to conclude that the growth of *Scirpus validus* takes place within an extremely short distance of the base, possibly as short as 2 or 3 mm.

Experiment 4

While the foregoing experiments were in progress, others were started, to test the effect of the medium upon the growing region. Pieces of rhizome were potted, practically at the surface of the soil, and placed under water. When the culms were well grown, the water was allowed to evaporate until the surface of the soil was exposed to the air. When the water level had fallen to about an

TABLE II

Variation in growth of *Scirpus validus* when changed from one medium to another

Pot	Water to air				Air to water				
	Accelerated		Retarded		Accelerated		Retarded		Unchanged
	No. of culms	Mm. per day	No. of culms	Mm. per day	No. of culms	Mm. per day	No. of culms	Mm. per day	No. of culms
A	1	16.0	1	22.0	1	0.5			1
B					1	4.5			
C			1	5.0	2	4.5 1.5			
D			1	11.0	1	1.0			1
F			1	12.0	1	1.5	1	1	
G					1	5.0			
AA	1	2.0	2	15.0 2.5	2	2.5 3.0			1
BB			1	24.0	2	1.0 2.0	1	4	
CC	1	42.0			2	3.0 2.5			
DD			1	17.5	1	2.0			
EE					2	1.0 6.5			
Averages		20.0		13.6		2.6		2.5	
Total nos.	3		8		16		2		3

inch below the surface of the soil, it was maintained at this height by regulating the amount of water in the surrounding vessel. This allowed the culms to grow in the air, while the roots had an abundant supply of water. All were grown under these conditions for a certain period, which differed for the different pots, after which they were again submerged. Careful measurements of the growth were made throughout the experiment, and at its close longitudinal sections were made in the regions which had grown under the different conditions, and measurements made of the distances between the diaphragms. The results are shown in the tables.

EFFECT ON GROWTH.—From table II it might be concluded that a change from water to air retards growth, and the reverse change accelerates it. Two facts, however, must be noted: (1) in some cases in the same pot one culm was growing faster after the change, while another was growing more slowly; and (2) the temperature was not controlled. Toward the latter part of the experiment an effort was made to keep a record of the variation in temperature. A recording thermometer was not available, consequently four thermometers were hung at different points among the plants and an average taken. As the greenhouse was supposed to be between 60–70° F., the readings were taken about 2:00 P.M. each day, merely to note any marked change in temperature. It was found, however, that the variation was too great and the readings too far apart to make the data of any value

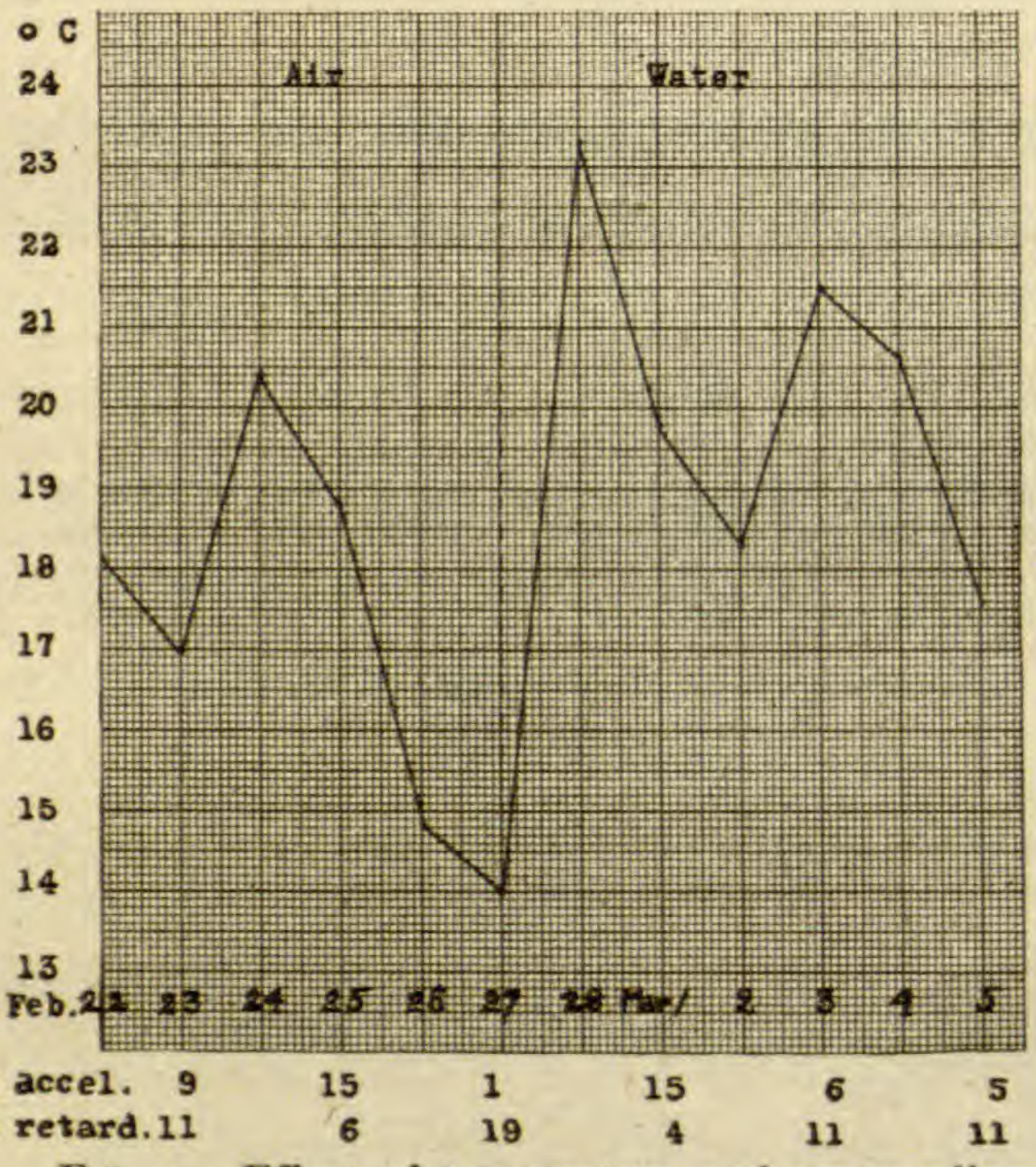

FIG. 1.—Effect of temperature and surrounding medium upon growth of culms of *Scirpus validus*.

except to show that the change from air to water was accompanied by a marked rise in temperature (fig. 1). This experiment indicates that water may not be as important a factor in the growth of *Scirpus validus* as temperature. This is probable because the growing region is protected from the surrounding medium by a very closely fitting sheath of scale leaves.

EFFECT UPON DISTANCE BETWEEN DIAPHRAGMS.—The distance was measured between many diaphragms and the average taken. As it was found that the diaphragm distance varied with the distance from the tip, the culms were divided into decimeter sections, so that the measurements taken in corresponding sections of the different culms, and in different sections of the same culm, might be compared.

TABLE III

DISTANCES IN MM. BETWEEN DIAPHRAGMS OF *Scirpus validus* GROWN IN WATER AND IN AIR

Pot	Culm no.	0–1 dm. from tip	1–2 dm. from tip	2–3 dm. from tip	3–4 dm. from tip	4–5 dm. from tip
A.	3		**3.5**		4.8	4.6
	5		2.6			
E.	2		**2.7**	**3.80**	**4.4**	**8.2*** / **3.0***
CC.	2	**2.5*** / **4.0***				
	3	1.6	3.6			
		2.4	3.0	3.85†	3.9†	
DD.	3	1.9*				
	4	1.6*				

* Two chambers in same section. † May extend over decimeter boundaries.

In table III the heavy type indicates parts grown in water, and the remaining figures indicate those grown in air. Read horizontally, the variation in a single culm, from tip to base, may be seen. Read vertically, the variations in the same region of the different culms are shown. As it was impracticable, from the data at hand, to calculate the rates of growth for the decimeter regions, it was not possible in this experiment to correlate the rate of growth with the distance between diaphragms.

EXPERIMENT 5

It was shown in experiment 4 that temperature control was very necessary; therefore in April 1916 experiment 5 was set up

in the greenhouse of the University of Chicago, using plants from the material started at the Missouri Botanical Garden in the fall.

TABLE IV

VARIATIONS IN RATE OF GROWTH AND STRUCTURE OF CULMS OF *Scirpus validus* UNDER DIFFERENT CONDITIONS OF MOISTURE AND HEAT

Culm no.	Region (mm. from tip)	Point at which change occurred (mm. from tip)	Growth (mm. per day)	No. of spaces	Layers in walls	Distance between dia-phragms†	No. of layers of palisades	Medium	Tempera-ture ° C.
E 2	132–172		6.00	4+	1	1.2	2	Water	35
	172–210		8.33			1.8			
	210–247		8.75	Many‡	1	2.5	2–0		
		247							
	247–290		6.14	Many	1–2	5.1	0	Air	30
E 3	50– 80		22.50			2.8		Air	30
	100–130		22.00	4+	1–2	3.6	2		
	142–172		30.00	4+	1–2	3.4	2		
	240–270		70.00			2.9			
		205							
	290–320		10.00	Many	3	3.2	0	Air	21
B 3	200–240		18.00			1.8		Water	35
	260–290		55.00			2.5			
	310–350		48.00	4+	1	3.0	2		
	400–450		21.66			4.1			
	500–550		20.00			4.6			
B 4	50– 90		15.33	4+	1	1.4	2	Water	35
		96							
	105–135		8.33	4+	1	1.8	2	Water	16
	160–182		27.00			3.0			
	200–240*					4.2			
	260–290*		29.50	4+	1	2.4	1		
		304							
	310–350		15.00	4+	1	2.8	1	Air	16
	400–450		20.00			2.8			
AA 3	100–140		17.50	4+	1	1.8	1	Air	16
		169							
	200–240		20.50			2.3		Water	16
	260–300		23.00			2.3			
	330–370*		23.25	Many	1–2	3.5	2		
		410							
	400–440		47.00			3.0		Water	30
	500–540		29.00			4.1			
	600–630		24.00			4.9			
DD 3	800–857		13.60	Many	1–2–3	6.1	0	Water	21±
		862							
	872–920		7.00	Many	1–2–3	6.4	0	Air	21±
DD 7	60–110		18.00	4+	1(?)	1.7	1–2	Air	21±
		119							
	125–175		39.00	4+	1(?)	1.7	2	Water	21±
	300–350		33.50	Many	1–2	3.5	2		
	400–450*		37.00			3.0			
		508							
	515–565		16.66	Many	1–2–3	3.4	2	Water	16
DD 8	44– 74		9.00	4+	1	1.7	2	Water	22±
	100–150*		21.50	4+	1–2–3	2.2	2		
		162							
	200–250		18.66	4+	1	2.7	2	Water	16

* Not observed for a period of 4 days.

† Distance between diaphragms in mm.

‡ "Many" indicates a number of fairly equal-sized spaces in contrast with 4 large spaces and several small ones.

The thermostats were kindly loaned by the Chemistry Department, and were regulated by Captain de Klotinski. The higher temperature vessel was kept within a degree of 35° C. for two weeks, and for the remainder of the experiment practically at 31° C. In the low temperature vessel a coil of pipe, carrying a stream of cold water, kept the temperature close to 15° C. for the first two weeks, and for the rest of the time about 16° C. The temperature in this vessel was not quite so constant as in the higher temperature thermostat, because of the varying water pressure. A third set of plants was allowed to grow without temperature control. When the change to air was made, the pots were transferred to beakers

TABLE V

RELATION BETWEEN RATE OF GROWTH AND DISTANCE BETWEEN DIAPHRAGMS IN *Scirpus validus*

Change	Culm no.	Rate	Distance
Water to air	E 2	Decreased	Increased
	B 4	Decreased	Increased
	DD 3	Decreased	Increased
Air to water	AA 3	Increased	Increased
	DD 7	Increased	Increased
Low to high temperature	AA 3	Increased	Decreased
High to low temperature	E 3	Increased	Decreased
	B 4	Decreased	Increased
	DD 7	Decreased	Increased
	8	Decreased	Increased

sunken to the rim in the water of the thermostats. Water was poured into the beakers to within an inch of the surface of the soil. The tops were covered with two pieces of glass, allowing the culms to project between them, and the crack was plugged with cotton wool. Close observation showed the temperature in the beakers to be practically the same as that of the water outside. Measurements were taken every 24 hours, and the rate of growth given for a region was usually the average for several days, thus eliminating the questioned stimulating effect of the change (**1, 9, 10, 14**). At the end of the experiment longitudinal and cross sections were made in the regions grown under the different conditions. The cross-sections were usually made at one end of a region

and the rest cut longitudinally, and therefore the results given in table IV are obviously not for exactly the same spot.

The number of changes was insufficient for reliable conclusions; also it must be remembered that under normal conditions there is a general tendency for the distance between diaphragms to increase from tip to base (see also B 3). Certain indications, however, are summarized in table V.

Discussion and conclusion

Growth.—*Region.*—So far I have found no reference to the region of growth in the stem of *Scirpus*. Pfeffer (**10**) refers to the basal region of growth in the leaves of *Canna* and *Tulipa* and in the internodes of grasses, and states that the "length of the zone is always small." He also mentions the careful protection of this zone. The same statements may be made for the zone of growth in the *Scirpus* stem; the extreme narrowness of the zone, however, is rather surprising. Growth in diameter was not studied.

Rate.—The results of the experiments were not perfectly harmonious; but in general there seemed to be a tendency toward an increase in rate with a change from air to water, and a decrease with the reverse change. It seems probable, however, that temperature was a more important factor than water.

Diaphragm distance.—From a study of table III it is evident that the variation between culms growing under the same conditions was greater than that between culms growing under different conditions of air and water, thus eliminating water as a direct factor in determining the distance between diaphragms. Its indirect effect was studied in experiment 5, and although the data were too scanty for positive statements, certain facts are rather significant. As there is a normal tendency for the diaphragm distance to increase from tip to base, the cases of increase after a change in environment may not be significant. Two cases of decrease occurred, however; one accompanying a change from high to low temperature, and the other following the reverse change. This would eliminate the temperature change as the direct factor. The fact that both of the cases of decrease in distance were associated with an increased growth rate is the important point. Also,

of the 29 cases of a change in rate of growth and diaphragm distance shown in table IV, 19 (64 per cent) showed an inverse relation between the two; 9 (24 per cent) showed a direct relation; and in 3 cases (10 per cent) an equal distance went with an increased rate. When we remember that the normal tendency is to increase the distance from tip to base, these last three cases really show an inverse relation which, added to the 19 preceding, make 22 (75 per cent) which show an inverse relation. Of these, 14 (49 per cent of total) show an increase downward, which coincides with the normal tendency, while 8 (27 per cent of total) show a decrease downward in opposition to the normal tendency, and therefore the more significant. These confirm the indication shown by the 49 per cent, and tend to establish an inverse relation between rate of growth and distance between diaphragms.

This is not what one would expect if the distance between diaphragms is considered to be brought about by the excessive growth, or stretching, of the intervening tissues. It is what one would expect, however, if, as was suggested in a former paper (13), diaphragms are due to certain cells retaining their power of division and growth, while those above and below them lose this power and are drawn out into arachnoid cells by the growth of the surrounding tissues; and also if the formative activities show a gradient from beginning to end of the growth of the stem. This suggests several interesting questions. Is there such a formative gradient? Would the respiration test show a gradual decline in metabolic changes, or would it follow the growth curve? Is it possible that, in averaging the diaphragm distances in a region, a shortening, corresponding to the rise in the growth curve, was overlooked? Is the peak of the growth curve due wholly to a stretching period? If so, would this stretching counterbalance the tendency to shorten the diaphragm distance with the rise in the growth curve?

AIR CHAMBERS.—*Scirpus validus* appears to start with four large chambers and a number of small ones. As the culm grows, the small ones increase in size, until many nearly equal-sized spaces are the result. The different culms may pass through these stages at different rates; therefore the same regions cannot be compared. Only two cases were noted in which a rather rapid

increase in the number of spaces occurred; in one, following a change from air to water, there was a marked increase within a distance of 15 mm.; in the other a noticeable increase occurred within 9 mm., following a change from low to high temperature. From these experiments there is no clear evidence that either water, temperature, or rate of growth has any effect upon the number or size of chambers produced.

PARTITIONS BETWEEN AIR CHAMBERS AND OUTER WALL OF STEM.—The changes in environment used in these experiments appear to have no effect upon the regular course of development of the partitions, which seems to be an increase in the number of layers from one to three. No observable difference could be noted in the outer wall of the stems.

PALISADE LAYERS.—The curious banding, which is sometimes seen in *Scirpus*, occurred in experiment 5. The albescent spaces and the basal region contained no palisades. The dark green portions contained two layers of palisades, and the pale green spaces one layer, or two with only part of the cells chlorophyllous. The environmental changes in the experiment seem to have no effect upon the development of palisades, and cannot be held responsible for the banded appearance.

Reduced atmospheric pressure

EXPERIMENTS

During February 1916 a series of experiments was started in the temperate house of the University of Chicago, to test the effect of low atmospheric pressure upon the air chambers of water plants. The apparatus is shown in fig. 2 and described in the legend. The temperature of the house was controlled by the general heating system, and a recording thermometer showed a variation of a degree about 20–21° C. The barometric pressure was obtained in experiment 1 from the records of a government instrument outside the greenhouse, and the figures were reduced to metric readings at 21° C. In experiments 2 and 3 the pressure readings were obtained from the barometer in the Botany Building, and corrected for temperature only. The pressure in the experimental

jar was read on the manometer. It varied somewhat on account of the variation in the flow of the city water supply used to produce the suction. Plants of *Scirpus validus*, selected from those started in St. Louis, and *Cyperus alternifolius* (?), already growing in the greenhouse, were used.

Experiment 1

This extended February 4–14. The pressure varied between 10 and 20 mm. of mercury. This pressure was chosen because air

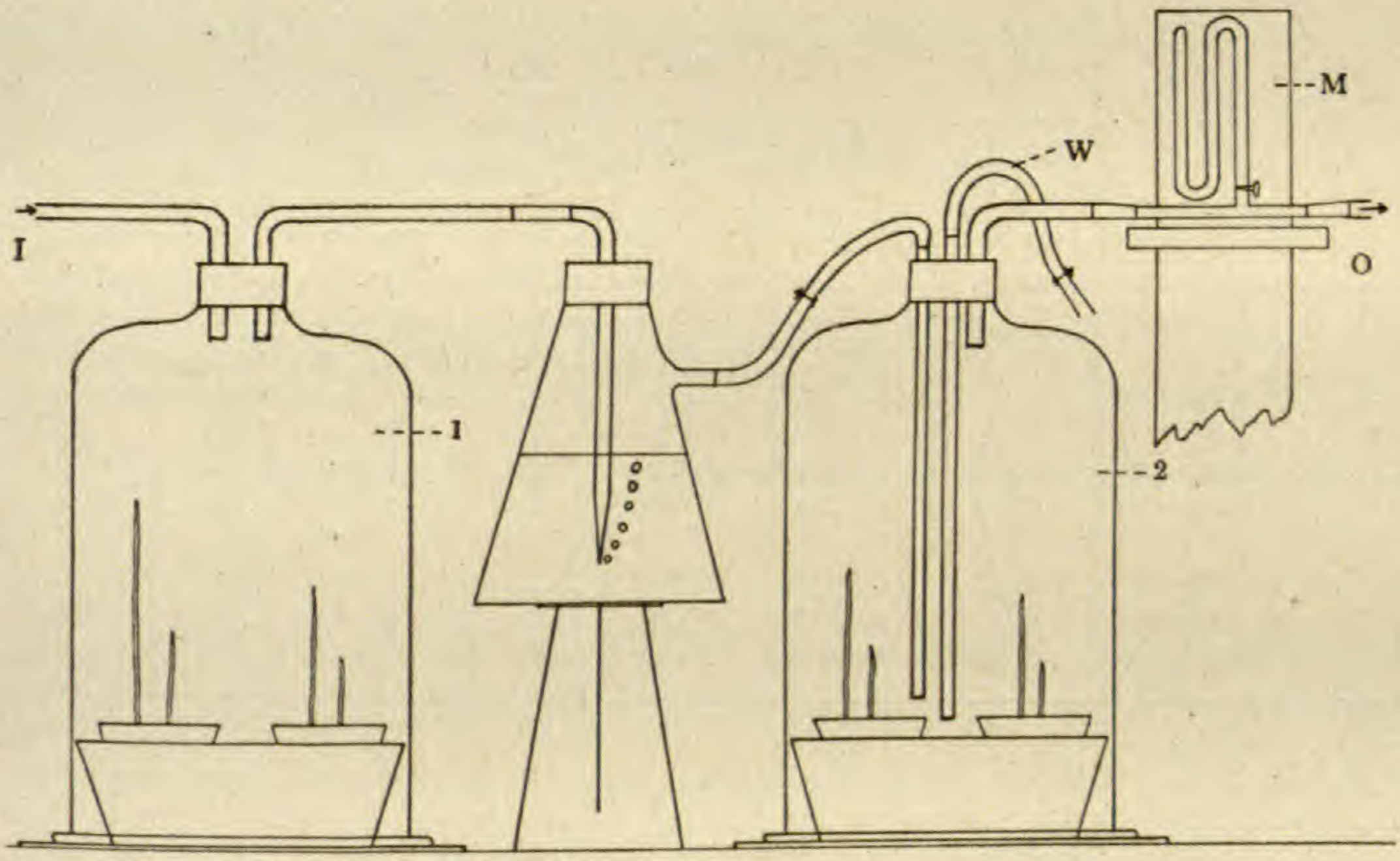

Fig. 2.—Apparatus to test effect of low atmospheric pressure: bell-jar 1, control; bell-jar 2, experiment; under each jar a pan of water containing pots of *Scirpus;* air entering at I and also around bottom of 1 (not sealed as was 2) passed out of 1 into flask where narrowed tube retarded flow and bubbles indicated rapidity of passage; in 2 air entered at bottom and escaped at top, thence through two catch bottles to pump operated by city water supply; *m*, manometer; *w*, tube for watering 2.

at 20 mm. pressure contains about as much oxygen as is dissolved in water (8). *Cyperus* would not grow at this pressure, and *Scirpus* alone was used. The culms were measured twice a day, and, after the close of the experiment, longitudinal and cross sections were made and measurements taken as in the previous experiments. Culms nos. 1 and 2 belonged to the control plant, nos. 3 and 4 to the experimental plant. Fig. 3 and tables VI and VII give the

results obtained. In fig. 3 the peak in the pressure curve at 5:30 (February 7) was due to the fact that the pinch-cocks were left open for a short time during watering. The second peak

TABLE VI

GROWTH AND STRUCTURE OF *Scirpus validus:* CULMS 1 AND 2 GROWN AT ATMOSPHERIC PRESSURE; CULMS 3 AND 4 GROWN AT 10–20 MM. PRESSURE

CULM NO.	REGION	DIAMETER IN MM.	WALLS IN MM.		SPACES		DIAPHRAGM DISTANCE IN MM.	GROWTH (MM. PER HOUR)
			Outer	Inner	Large	Small		
1.....	45–71	.....	.....	.....	.....	.....	.....	.....
2.....	45–71	2.00	0.168	0.050	4	6	2.36	0.51
3.....	45–71	.....	.....	.....	.....	.....	.....	.....
4.....	45–71	1.57	0.134	0.054	4	8	3.92	0.32
1.....	71–100	.....	.....	.....	.....	.....	.....	.....
2.....	71–100	1.89	0.156	0.050	4	10	2.55	0.85
3.....	71–100	2.25	0.103	0.060	15	9	3.50	0.53
4.....	71–100	1.58	0.119	0.052	7	9	4.05	0.49
1.....	100–124	.....	.....	.....	.....	.....	.....	.....
2.....	100–124	2.15	0.108	0.050	7	8	3.19	1.35
3.....	100–124	2.38	0.150	0.050	15	7	5.60	0.51
4.....	100–124	1.73	0.103	0.090	12	7	3.90	0.21
1.....	124–175	2.50	0.050	0.065	17	16	6.08	.....
2.....	124–175	2.25	0.128	0.070	7	9	3.73	1.39
3.....	124–175	2.50	0.113	0.090	16	7	5.18	0.36

TABLE VII

INCREASE IN DIAPHRAGM DISTANCE IN MM.

Region	No. 4 over no. 2	No. 3 over no. 2
45–71.....	0.56	.....
71–100.....	1.50	0.95
100–124.....	0.71	2.41
124–175.....	.....	1.45

cannot be definitely accounted for by the data at hand. It is very probable that in these two cases, as well as in two others, an adjustment was made at once, but there are no records of the fact, such as occur later. The dotted line, therefore, is probably the more correct one.

Experiment 2

This experiment extended February 14–28. It was set up in the same apparatus as was experiment 1; the pressure, however, started at 35 mm. and varied between 20 and 40 mm. of mercury. *Cyperus* would not grow at this pressure; consequently the results

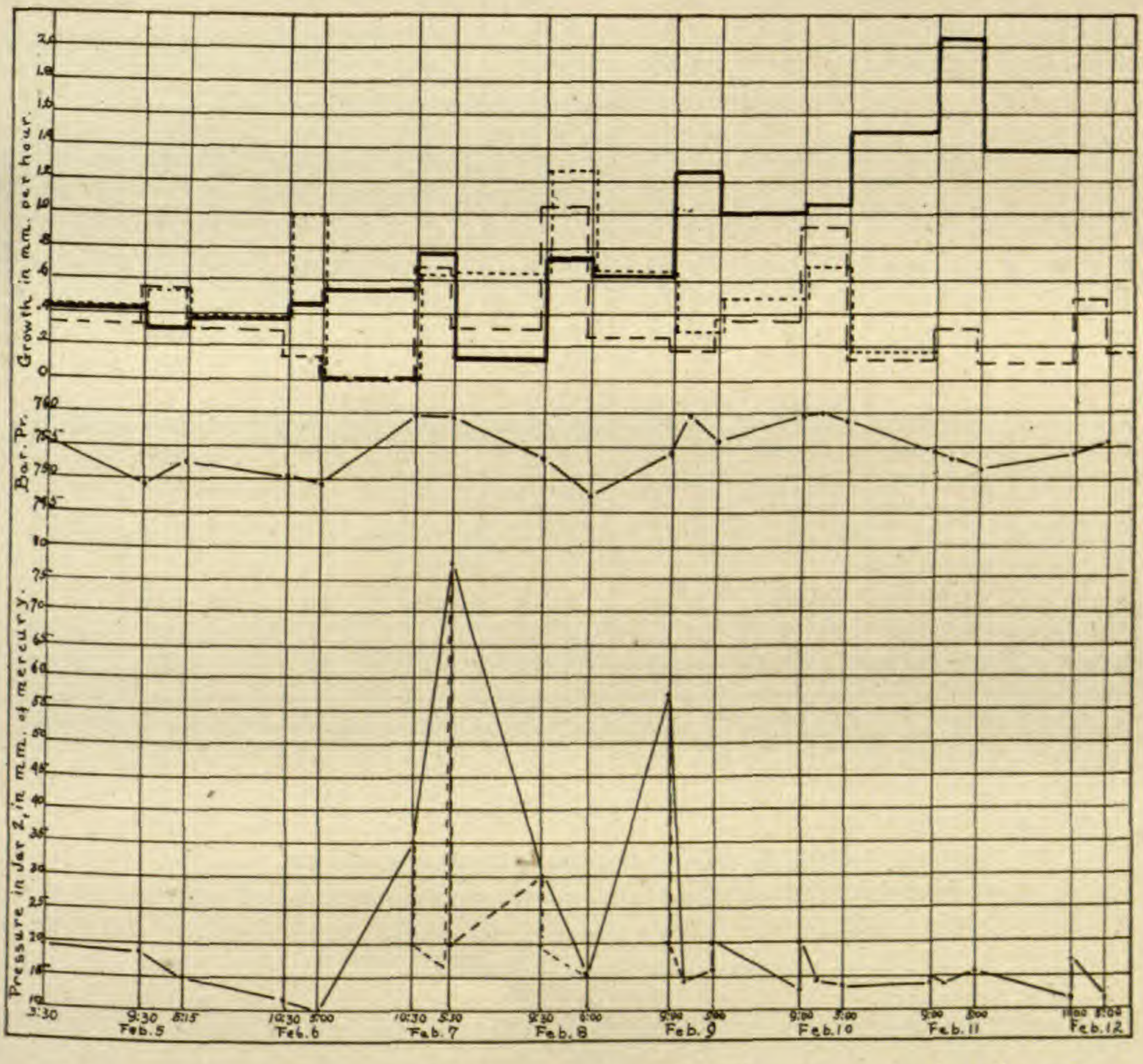

FIG. 3.—Growth curves: solid line, no. 2; dotted line, no. 3; broken line, no. 4; curves 2 and 3 cease when culms reached top of jar.

are given for *Scirpus* only. The quantity of air passing through the jars was measured and found to be about 1500–2100 cc. per hour. At times it exceeded this amount, but the flow was kept fairly constant. The experimental culms were nos. 3 and 4, and the controls nos. 1 and 2. Tables VIII and IX give the results.

TABLE VIII

GROWTH AND STRUCTURE OF *Scirpus validus:* CULMS 1 AND 2 GROWN AT ATMOSPHERIC PRESSURE; CULMS 3 AND 4 GROWN AT 20–40 MM. PRESSURE

CULM NO.	REGION	DIAMETER IN MM.	WALLS IN MM.		SPACES		DIAPHRAGM DISTANCE IN MM.	GROWTH (MM. PER HOUR)
			Outer	Inner	Large	Small		
1.....	0– 10	0.885	0.14	0.05	3	5	.....	.....
2*.....	0– 10	0.895	0.11	0.04	4	5	.....	.....
3.....	0– 10	0.910	0.11	0.05	2	2	.....	.....
4.....	0– 10	1.010	0.08	0.05	4	6	.....	.....
1.....	10– 30	1.260	0.17	0.03	4	4	1.630	0.48
2*.....	10– 30	1.775	0.14	0.04	4	9	0.085	.....
3.....	10– 30	1.545	0.13	0.05	4	7	2.170	0.37
4.....	10– 30	1.550	0.14	0.06	5	11	3.100	0.24
1.....	30– 65	1.450	0.18	0.09	4	8	1.750	0.68
2*.....	30– 65	1.830	0.15	0.06	12	9	2.150	.....
3.....	30– 65	1.910	0.14	0.05	4	8	3.400	0.55
4.....	30– 65	1.800	0.13	0.05	5	14	2.950	0.49
1.....	65–105	1.600	0.14	.....	4	8	2.320	0.83
2*.....	65–105	2.000	0.12	0.06	16	10	2.030	.....
3.....	65–105	2.155	0.15	0.07	4	15	2.980	0.57
4.....	65–105	2.050	0.13	0.09	5	16	3.180	0.47
1.....	105–130	.....	.....	.....	.....	.....	.....	.....
2.....	105–130	1.940	0.13	0.05	18	10	2.300	.....
3.....	105–130	2.255	0.15	0.08	4	15	3.270	0.83
4.....	105–130	1.875	0.11	0.07	11	12	3.480	0.95

* Culm 2 grew 110 mm., before the experiment, under practically the same conditions as the control; the measurements are used with this fact in mind.

TABLE IX

INCREASE IN DIAPHRAGM DISTANCE IN MM.

Region	No. 3 over no. 1	No. 3 over no. 2	No. 4 over no. 2
10– 30.....	0.54	2.085	3.015
30– 65.....	1.65	1.250	0.800
65–105.....	0.66	0.950	1.150
105–130.....	.....	0.970	1.180

EXPERIMENT 3

This experiment was set up on February 28 under the same conditions as experiments 1 and 2, except for the pressure, which was kept between 60 and 80 mm. of mercury. The experiment was discontinued on March 4 because the culms had reached the top of the jars. At the end of the experiment the manometer

showed a trace of moisture, and was found, upon being tested with the air pump, to register 2 mm. too low. As the variation in pressure had been 20 mm., it was not thought necessary to correct

TABLE X

GROWTH AND STRUCTURE OF *Scirpus validus:* CULM 1 GROWN UNDER ATMOSPHERIC PRESSURE; CULM 2 GROWN UNDER 60–80 MM. PRESSURE

CULM NO.	REGION	DIAMETER IN MM.	WALLS IN MM.		SPACES		DIAPHRAGM DISTANCE IN MM.	GROWTH (MM. PER HOUR)
			Outer	Inner	Large	Small		
1.....	95–135	1.85	0.130	0.058	7	17	2.50	0.62
2.....	95–135	1.79	0.123	.....	4	15	2.36	0.52
1.....	135–173	1.86	0.133	0.065	12	12	2.41	0.80
2.....	135–173	1.95	0.135	0.082	7	15	2.29	0.89

TABLE XI

INCREASE IN DIAPHRAGM DISTANCE IN MM.

Region	No. 1 over no. 2
30– 59*	0.39
95–135	0.19
135–173	0.12

* Before the experiment.

TABLE XII

DIAPHRAGM DISTANCE AND RATE OF GROWTH OF *Scirpus validus* UNDER REDUCED ATMOSPHERIC PRESSURE

Experiment no.	Culm no.	Average distance between diaphragms in mm.	Average growth rate in mm. per hour
I.....	2*.....	2.96*	1.02*
	3.....	4.76	0.43
	4.....	3.96	0.32
II.....	1*.....	1.90*	0.84*
	3.....	2.96	0.62
	4.....	3.18	0.44
III.....	1*.....	2.46*	0.70*
	2.....	2.33	0.68

* Control culms grown under greenhouse conditions.

for this error. On the average 5 liters of air passed through the apparatus per hour. Culm 1 was the control and culm 2 the experimental one. Tables X, XI, and XII give the results.

Experiment 3 consisted of only two culms; therefore the disagreement found in the other experiments between the culms in the same pot is not noticed here.

Discussion and conclusions

Atmospheric pressure and growth.—Much work, with conflicting results, has been done upon the effect of varying degrees of atmospheric pressure upon growth. There is a rather general agreement that a certain decrease in pressure accelerates growth, but a difference of opinion as to whether this is due directly to diminished pressure or to a decrease in the partial pressure of the oxygen (**8, 11, 15**), also whether increased growth in water is due to decreased oxygen pressure or to some other factor.

Low pressure (10–20 mm. in experiment 1) seemed to have a general depressing effect upon growth in *Scirpus*, as will be seen in fig. 3 and table XII. The curve shows that this effect was not constant, however, and that growth did not follow the variations in pressure. In general there is greater growth in both control and experiment during the day. The graphs for experiments 2 and 3 show this last point somewhat more clearly, and also show the closer agreement of the two curves as the pressure in the experiment was increased, but they are not sufficiently striking to merit inclusion in this report. The power to grow fairly well with such a small supply of oxygen evidently enables *Scirpus* to grow in very poorly aerated situations.

Diaphragm distance.—If low atmospheric pressure had any effect upon distance between diaphragms, there would be a progressive increase, or decrease, in the experimental culms as compared with the control, because there is a normal tendency to increase the distance from tip to base. Tables VII and IX show that, while the experimental culms had a greater diaphragm distance throughout than the control, this did not increase progressively, but varied in the different regions. In experiment 3, however, the results were different. Before the experiment the control had the greater diaphragm distance (1.61–1.22 mm.). This persisted, but in a diminishing amount (table XI), which really means a progressive increase in the experimental culm. It is

possible that the greater diaphragm distance found in the experimental culms may have been due only indirectly to decreased pressure, through its effect upon growth. In every instance (except region 95–135, experiment 3) an increase in the diaphragm distance was correlated with decreased growth rate. This agrees with the results reported earlier in this paper.

NUMBER OF AIR CHAMBERS.—In reviewing the literature one finds a rather general opinion that air spaces increase with the amount of water in the substratum (**2, 3, 7, 10, 12, 16**). Recent work by FOLSOM (**6**) on *Ranunculus*, however, has shown that while the aerenchyma of the stem varied directly with the amount of water in the soil, that of the root showed no such constant relation, and in some cases even varied inversely. Various functions have been attributed to the air-containing tissue by different authors. Some consider it as "floating tissue" (**2, 9, 10**), while others consider it a means of oxygen supply, giving a lack of oxygen as its direct cause (**7, 10, 12**). On the other hand, WIELER thinks it has no function, and attributes its formation to the direct stimulus of water contact. DEVAUX (**5**) thinks that the hypertrophy of lenticels found in water and moist air is due to a checking of the transpiration, a factor which apparently has not been tested in connection with the formation of air spaces, although suggested some time ago by COWLES (**4**).

If low oxygen pressure is the cause of increased air spaces, it is rather strange that in FOLSOM's experiments the roots, which under any condition are farther from the oxygen supply than the stem, should show either inconstant increase or a positive decrease in aerenchyma with increase in water content of the soil. In experiment 3 there was a small increase in the total number of air chambers in the experimental culm, at the same time that the control remained the same. On the other hand, experiments 1 and 2 give evidence of a greater variation in total number of spaces and in their uniformity in size between the culms under like conditions than between the experiment and the control. Contrary to expectation, therefore, low atmospheric pressure appears to have no effect upon these two characters in *Scirpus*.

INNER AND OUTER WALLS.—The records show a close similarity in the width of the outer solid tissue of the culms of the control and of the experiment. This is also true for the partitions between the chambers.

PALISADES.—In experiment 1, one control culm developed one layer of palisades and the other two; while in experiment 2 the same thing occurred in the experimental culms. In the third experiment both culms had two layers. Atmospheric pressure seems therefore to have no effect upon palisade tissue.

EXPERIMENT 4

This experiment extended from April 11 to May 10. The apparatus used was the same as in the previous experiments, except that a tube dipping into a dish of mercury was used to indicate the pressure instead of the regular manometer. *Cyperus alternifolius* (?) was used in this and the following experiment instead of *Scirpus*. The time was divided into five periods, during which an effort was made to keep the pressure at 1/6 atm. (130± mm.), 1/3 atm. (250± mm.), 1/2 atm. (380± mm.), 3/4 atm. (570± mm.), and 5/6 atm. (630± mm.) respectively. The pressure varied very greatly, probably because the city water supply varied more at this time than earlier in the year. Culms 1, 2, 6, 7, and 8 were the control, and nos. 3, 4, 5, and 9 the experimental culms. Nos. 1, 2, 3, and 4 started before the experiment and were not used in the comparison. Culms 3 and 4 when placed at 1/6 atm. grew very little and ceased growth in 3–4 days. At the end of a week the pressure was raised to 1/3 atm., but without effect upon these culms. Toward the end of this period no. 5 started to grow slowly. At the same time no. 6 started in the control. With the rise to 1/2 atm. no. 5 grew better, but still kept behind no. 6. The rates approached each other very closely when the pressure rose to 3/4 atm. During this period no. 7 began to grow in the control, and as it followed very closely the growth curve of no. 6 (which had grown very tall) it was used for comparison with no. 5. At 5/6 atm. no. 7 still maintained a higher rate than no. 5, which by this time had about reached the limit of its growth. About the middle of this period no. 8 started in the control, and no. 9

in the experiment, and the graphs for nos. 7, 8, and 9 are so nearly alike that it is evident that this amount of pressure has the same effect on the growth of *Cyperus* as full atmospheric pressure.

After the experiment was over, cross-sections were made at the same distances from the top in the two sets of culms, but no evidence that diminished pressure had any effect upon the air spaces could be observed.

Experiment 5

This experiment was set up May 10, using the same apparatus as in the last experiment. The pressure was kept for 3 days at about 1/2 atm. (380± mm.) and for 2 weeks at about 7/10 atm. (530± mm.), after which it varied around 1/3 atm. (250± mm.) for 6 days. Growth was not measured, but after May 31 cross-sections were made. A careful study of these show that at 30 mm. from the base the experimental culm was a little more lacunose than the control, at 50 mm. still more so, but at 70 mm. it showed less difference. At 95 mm. the experimental culm ended in the usual tuft of leaves, while the control grew much higher, and at 95 mm. showed a structure exactly like that of the experimental culm at 50 or 70 mm. The shorter culm evidently had not differentiated as much as the longer one, and the difference in the sections was therefore only apparent and not due to the effect of diminished pressure.

From these two experiments one must conclude that, although atmospheric pressure reduced below 630± mm. had a retarding effect upon the growth of *Cyperus*, there is no evidence that it had any effect upon the air spaces.

Summary

1. The zone of growth of *Scirpus validus* is very short, possibly 2–3 mm.

2. The direct contact of the surrounding medium with the growing region is prevented by a closely fitting sheath of scale leaves.

3. In these experiments the rate of growth, in general, was increased by a change from air to water, and from low to high

temperature; while the reverse changes resulted in a decrease in the rate.

4. Temperature seemed to be a more important factor than water.

5. The increase in diaphragm distance which followed a change from water to air, and from high to low temperature, did not seem to be sufficiently great to be considered a direct result of the change, inasmuch as there is a normal tendency to increase from tip to base.

6. There appeared to be an inverse relation between diaphragm distance and rate of growth.

7. Environmental conditions may influence diaphragm distance by their effect upon growth.

8. A decreased growth rate would indicate a lowering of the vital activities of the plant, and would result in the formation of fewer diaphragms, thus increasing the distance between them.

9. This decreased vitality was shown normally in the decrease in growth rate toward the close of the growth period, and was accompanied by an increase in diaphragm distance.

10. This plant grew fairly well under 10–20 mm. pressure, while under 60–80 mm. pressure there was almost as good growth as under normal pressure.

11. There appeared to be an increase in diaphragm distance at low pressures. Apparently this was due to the retarding effect of diminished pressure upon growth.

12. Lowered pressure appeared to have no effect upon (1) the total number of air chambers or their size, (2) the thickness of the mass of tissue on the outside of the stem or of the partitions between the chambers, and (3) the number of palisade layers.

13. These experiments lead one to conclude either that water with its low oxygen content is not the direct cause of the air spaces in aquatics, or that *Scirpus validus* is a very non-plastic organism, retaining its characteristic growth and structure under wide variations in environmental conditions.

14. A lowering of the atmospheric pressure below 630± mm. had a retarding effect upon the growth of *Cyperus alternifolius* (?), but there is no evidence that it had any effect upon the air spaces.

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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

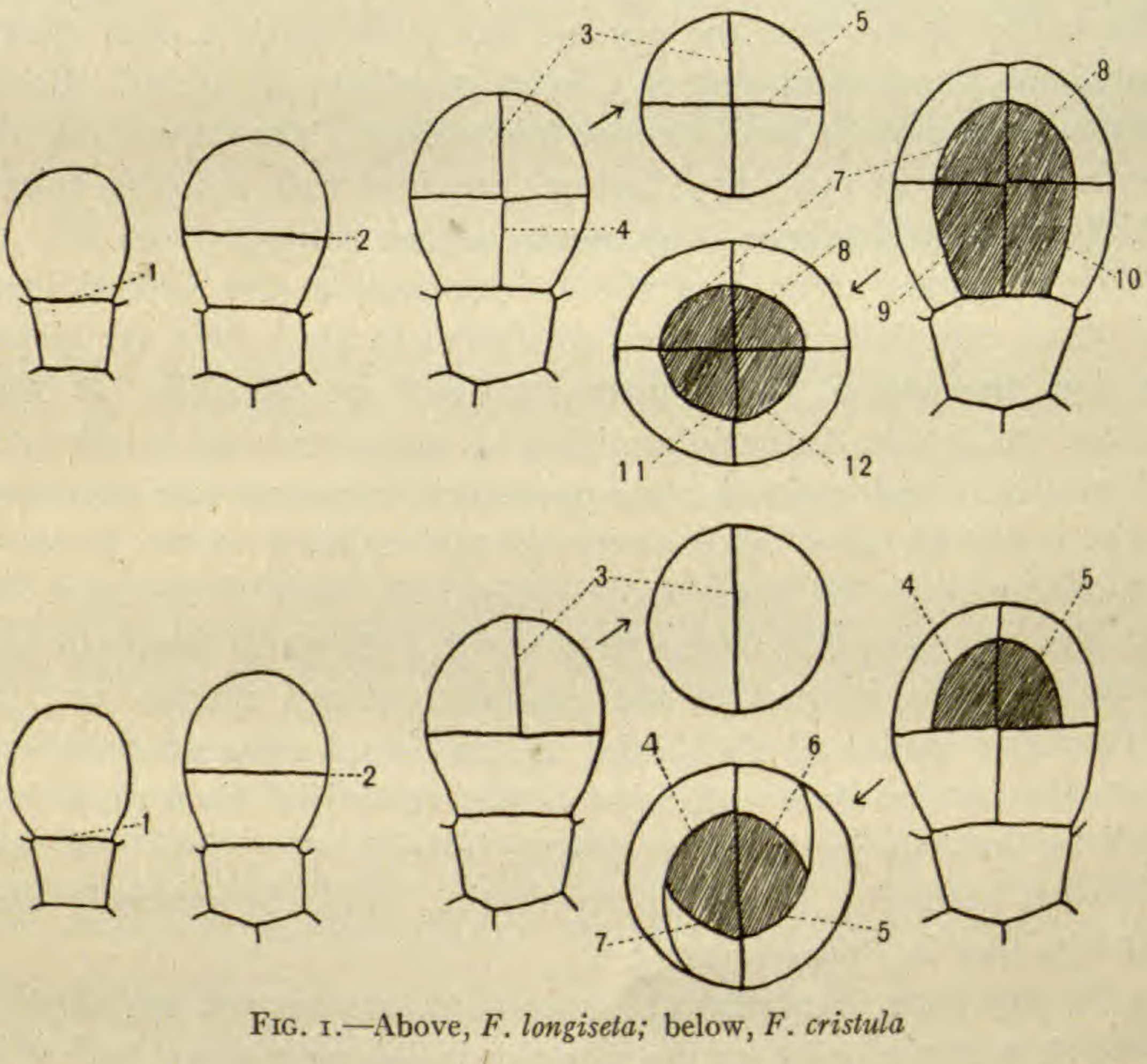

FIG. 1.—Above, *F. longiseta;* below, *F. cristula*

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.

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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.

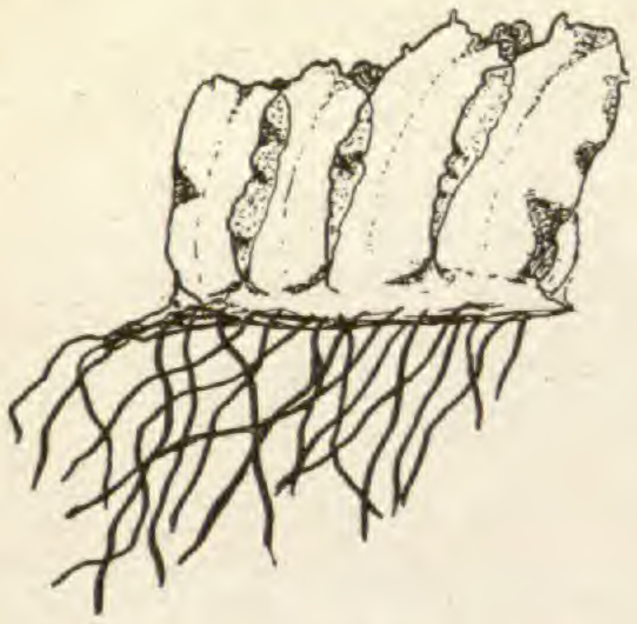
FIG. 23.—Archegonium initial; ×790.

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.



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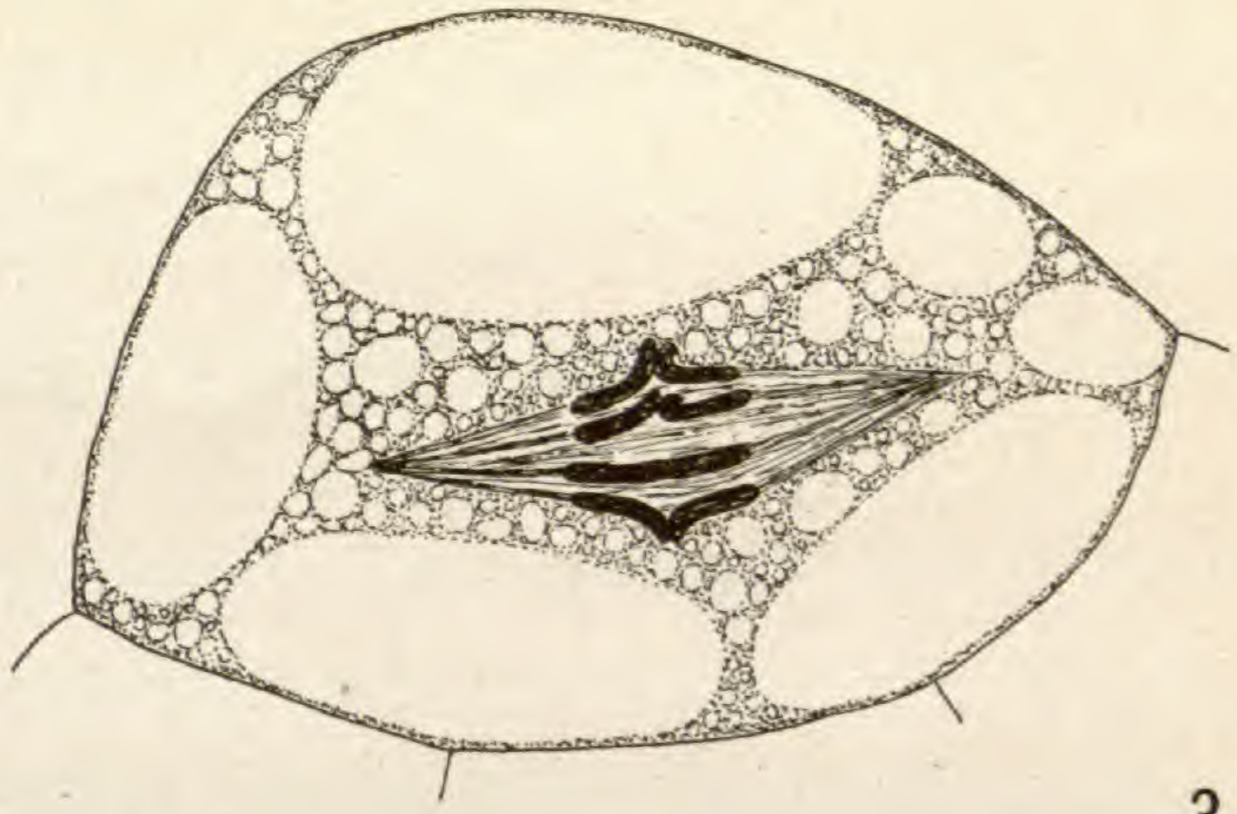

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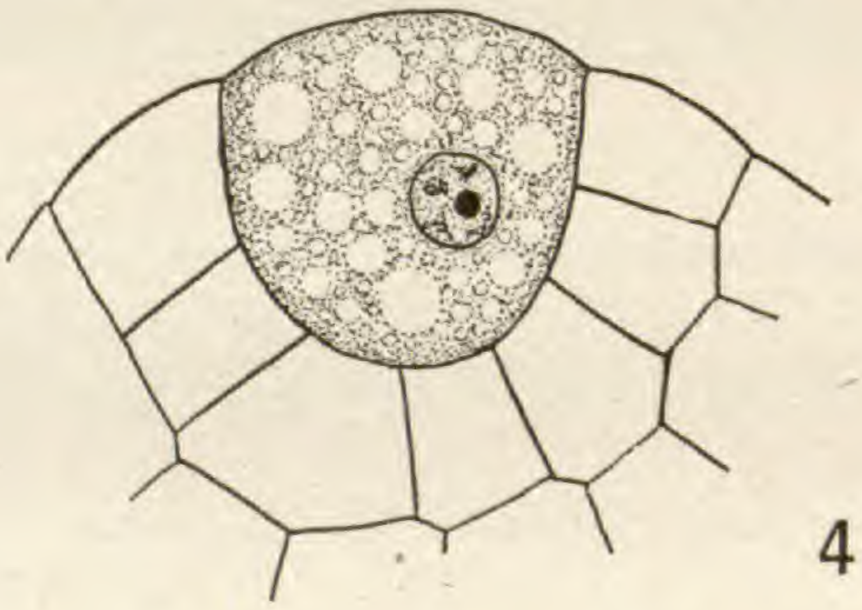

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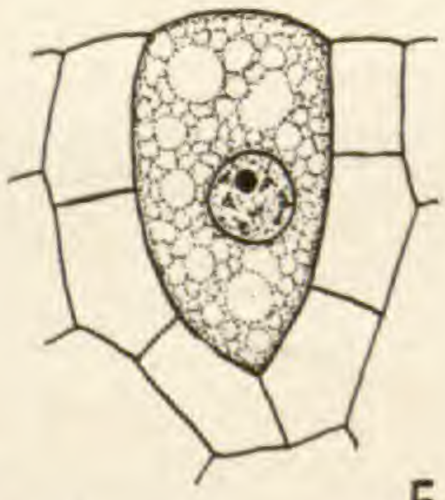

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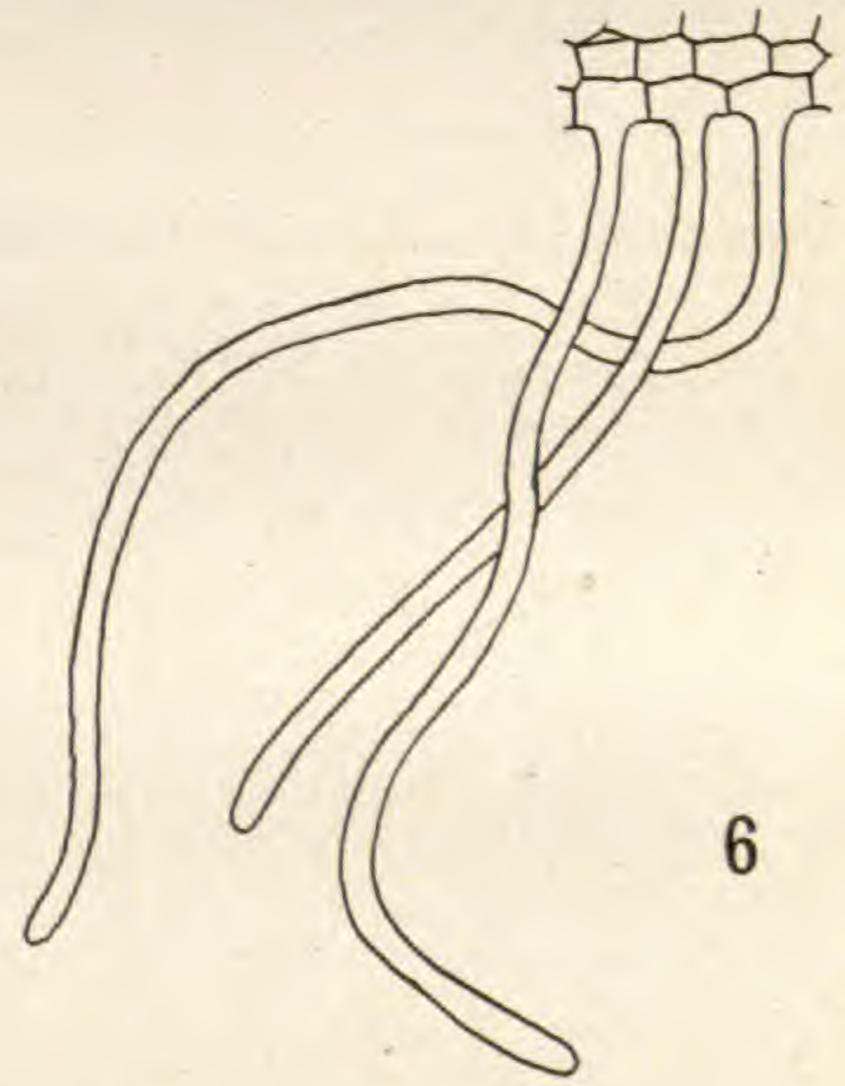

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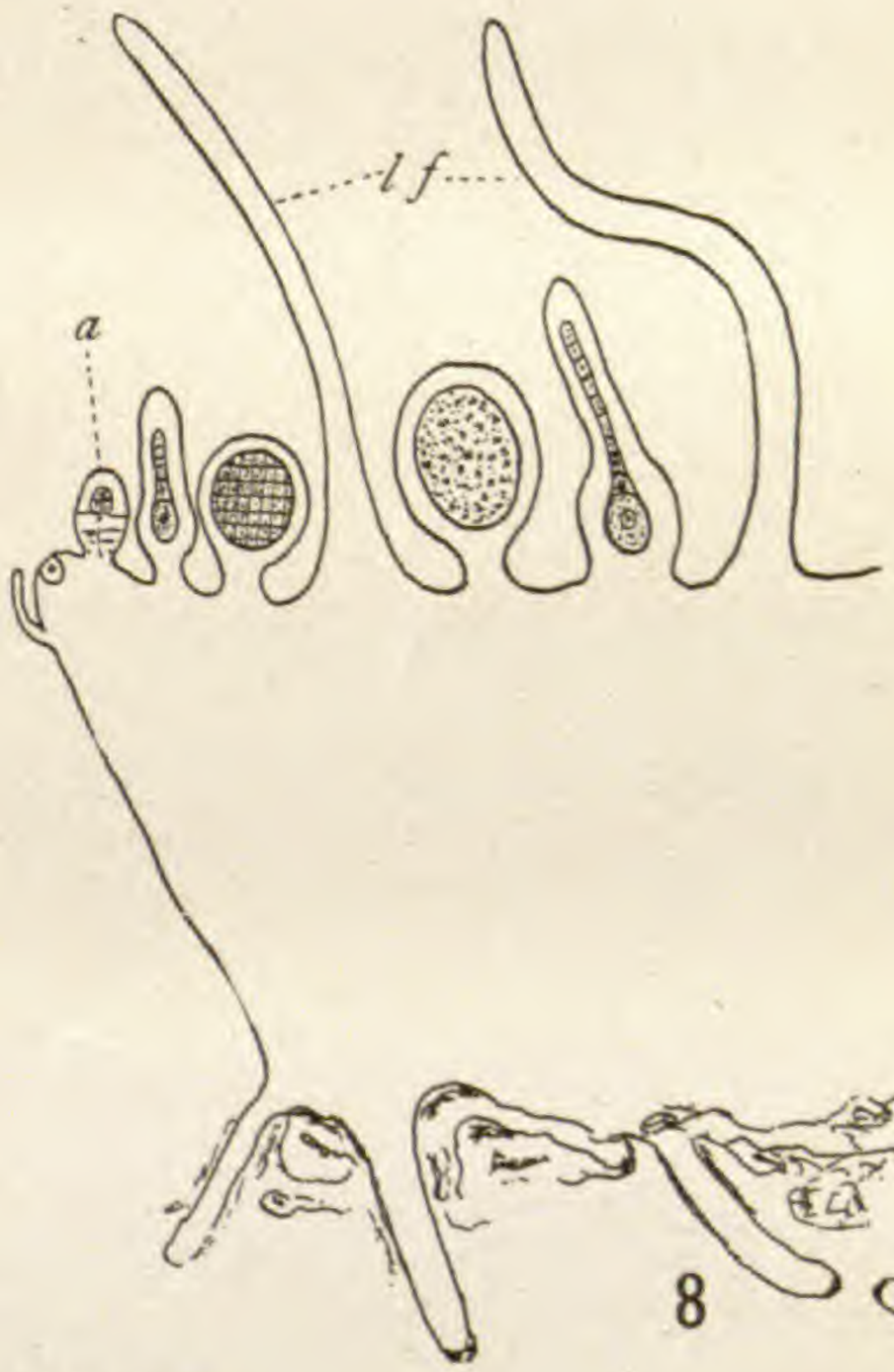

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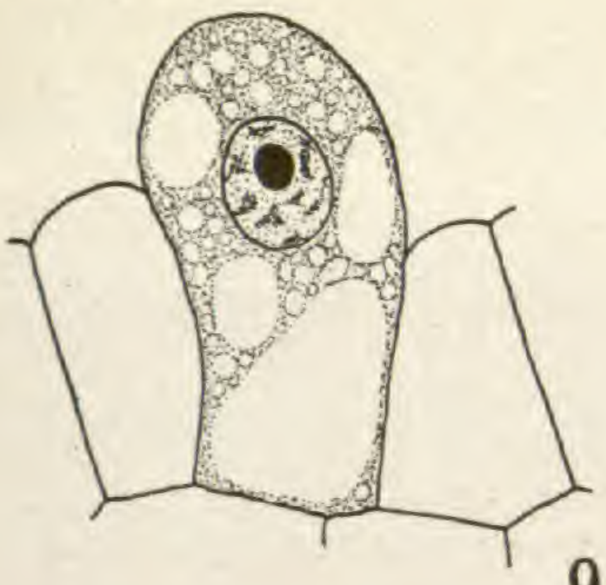
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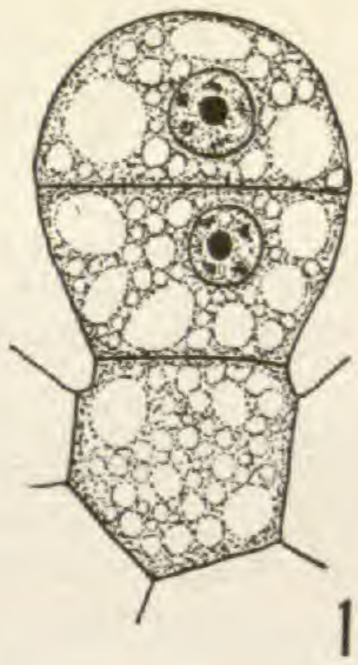
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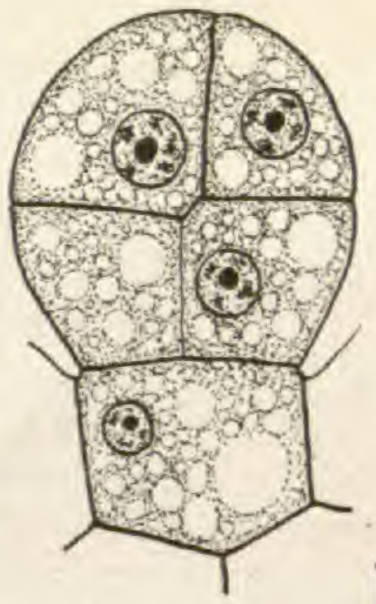
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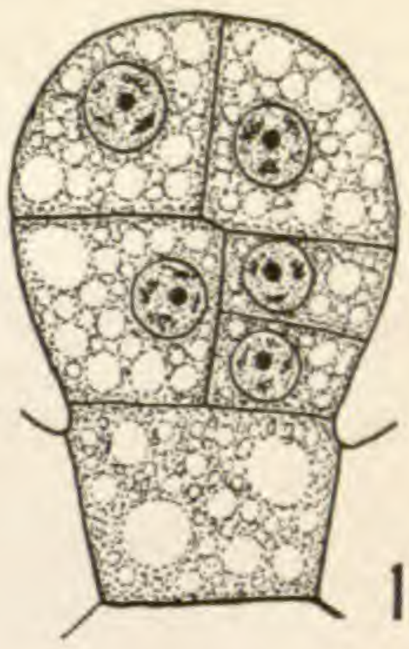
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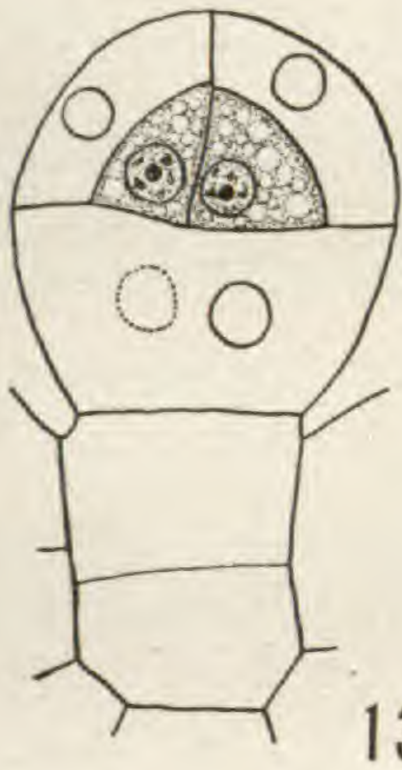
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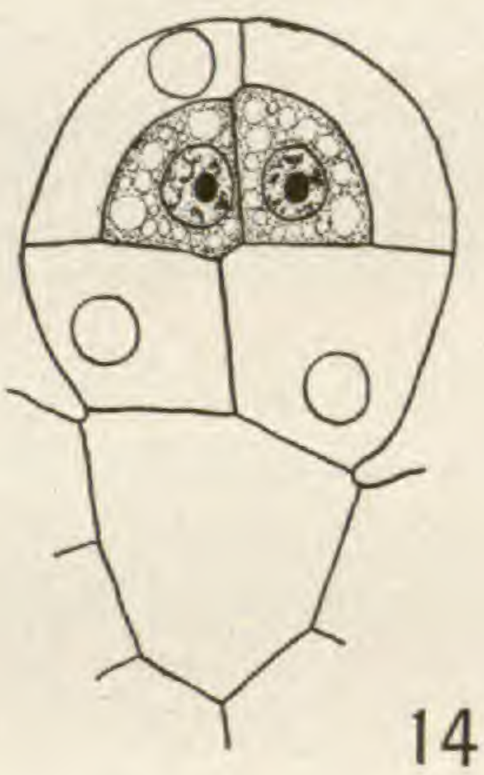
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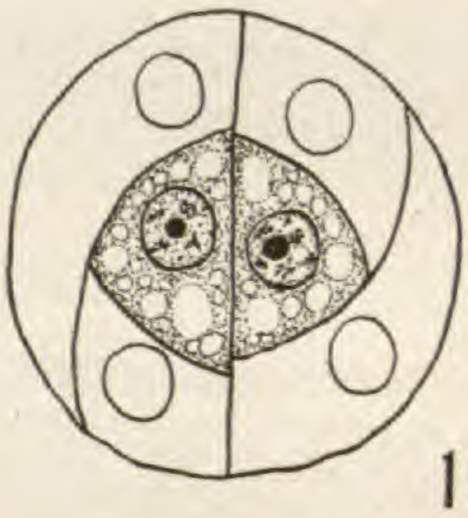
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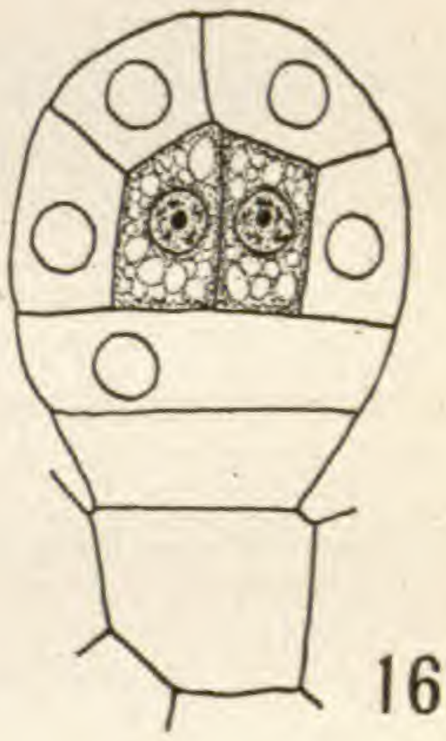
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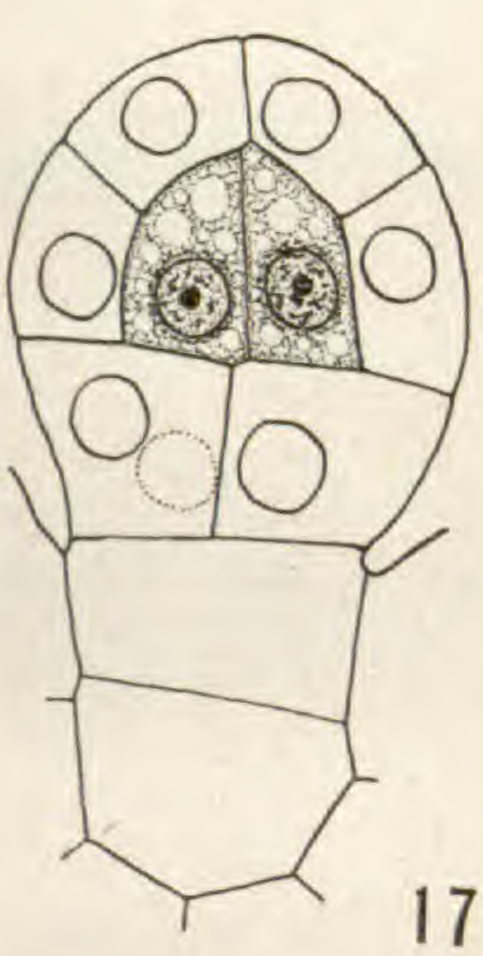
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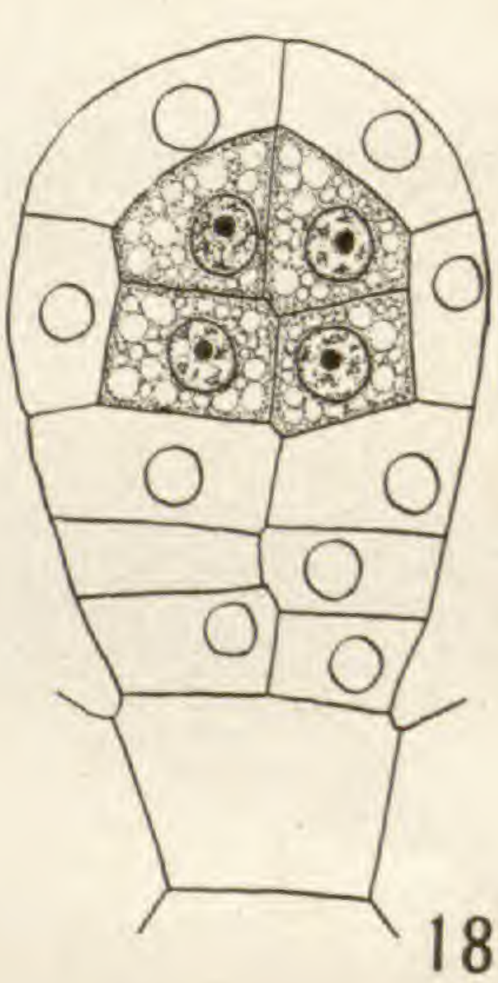
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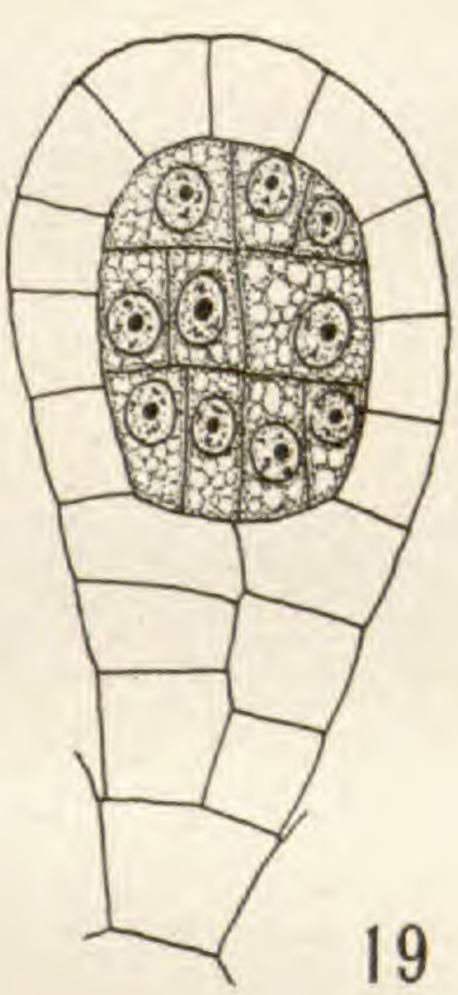
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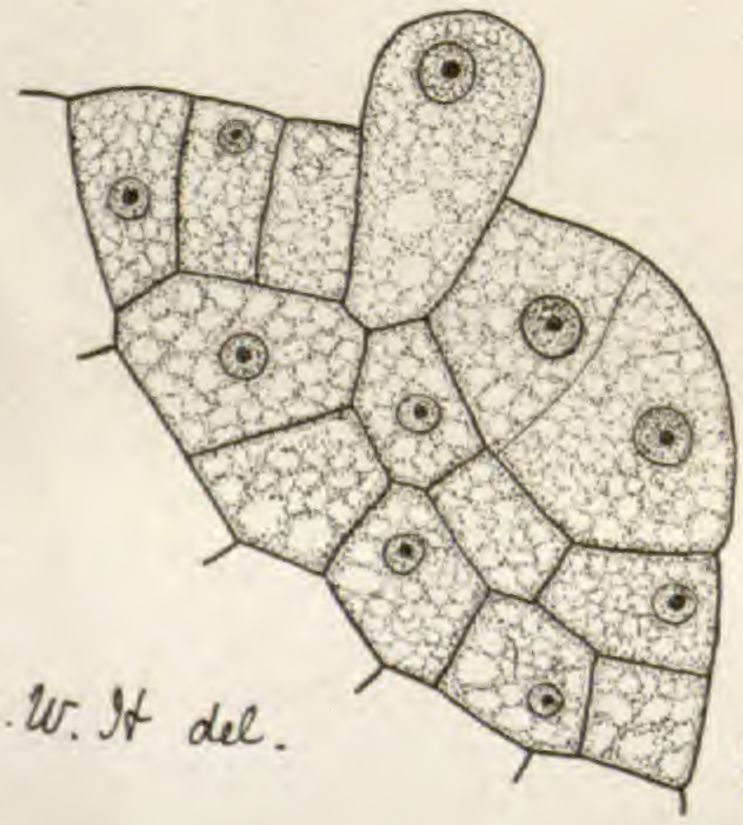
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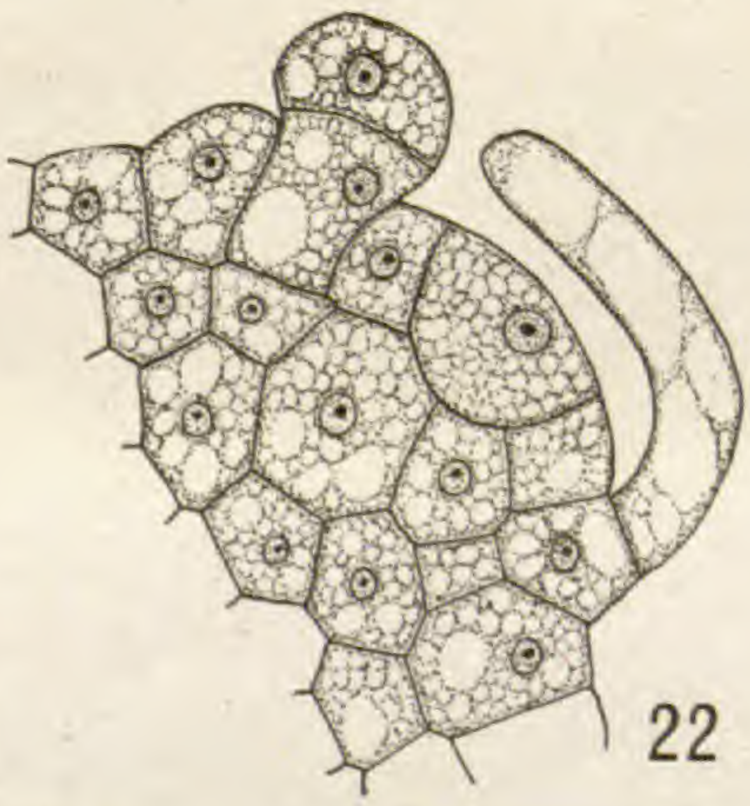
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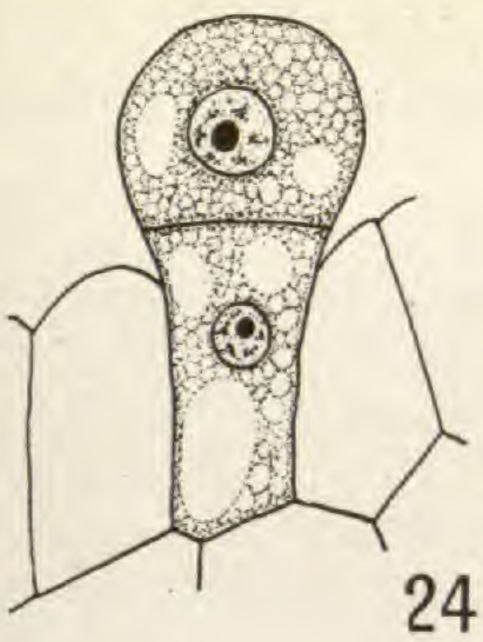
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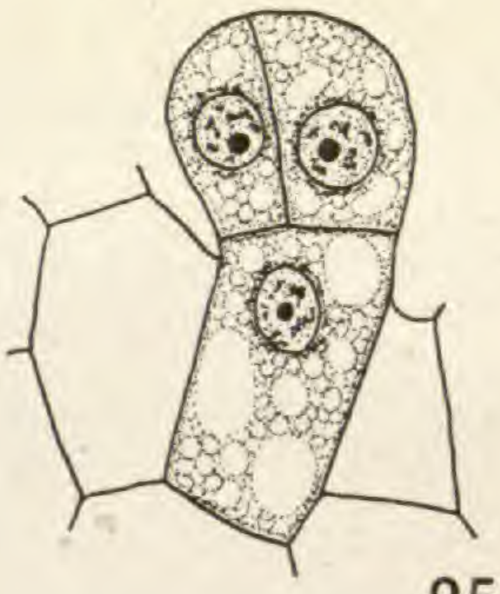
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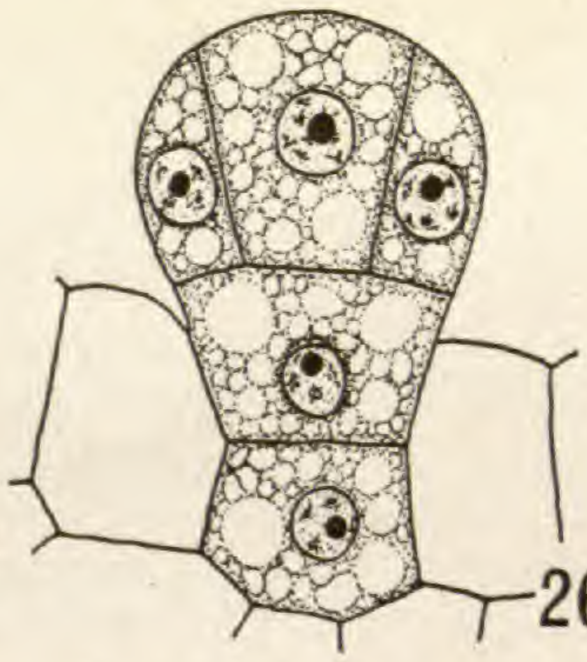
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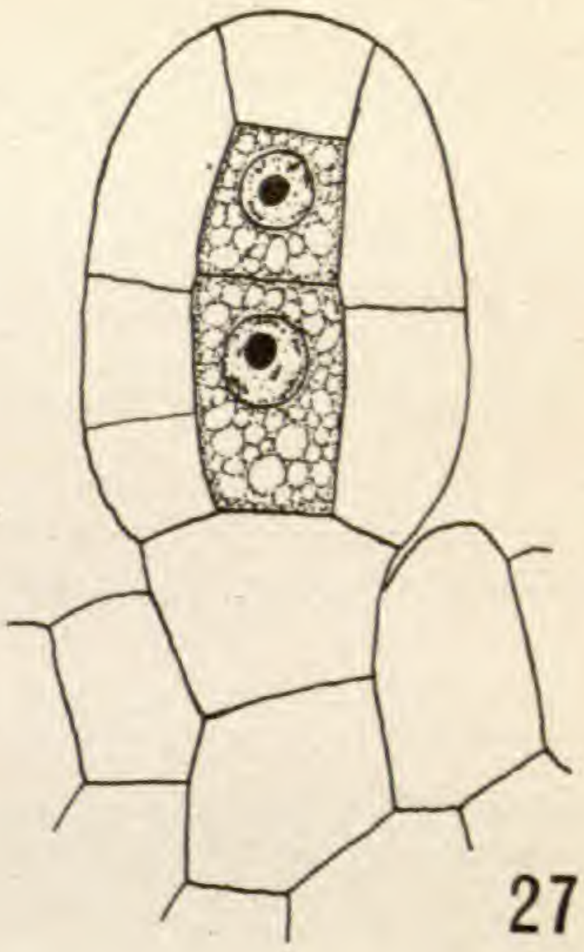
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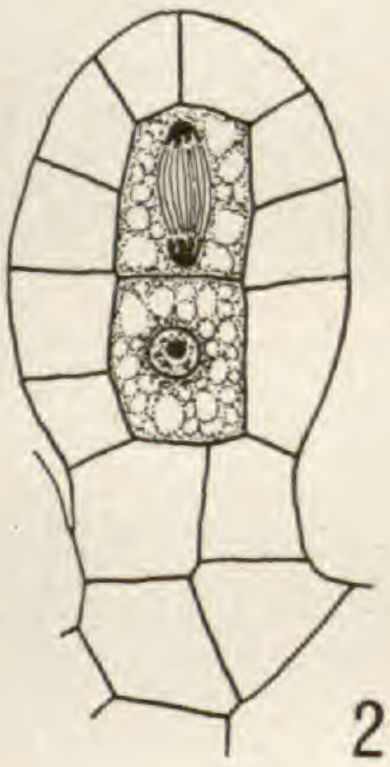
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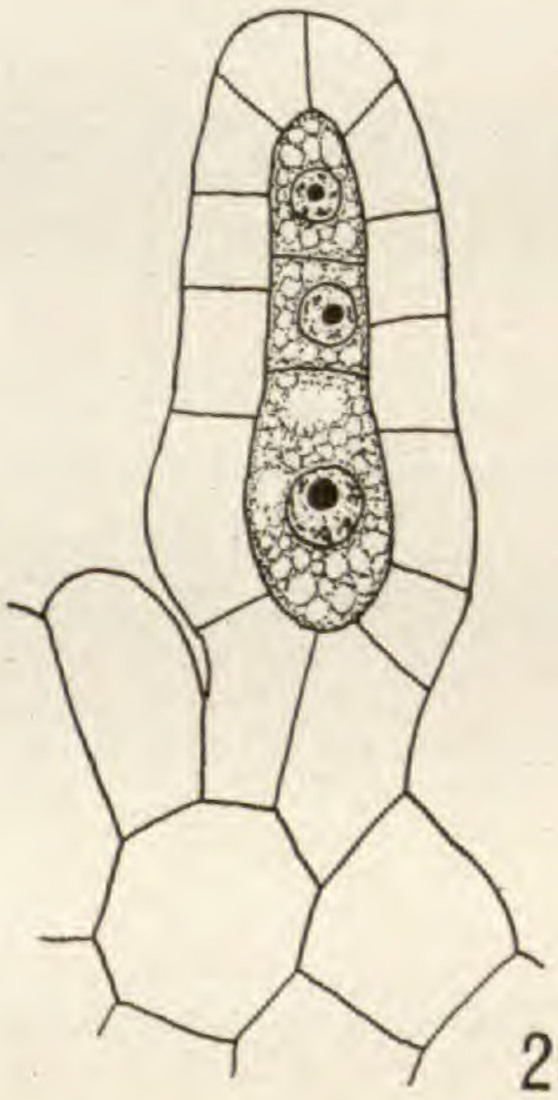
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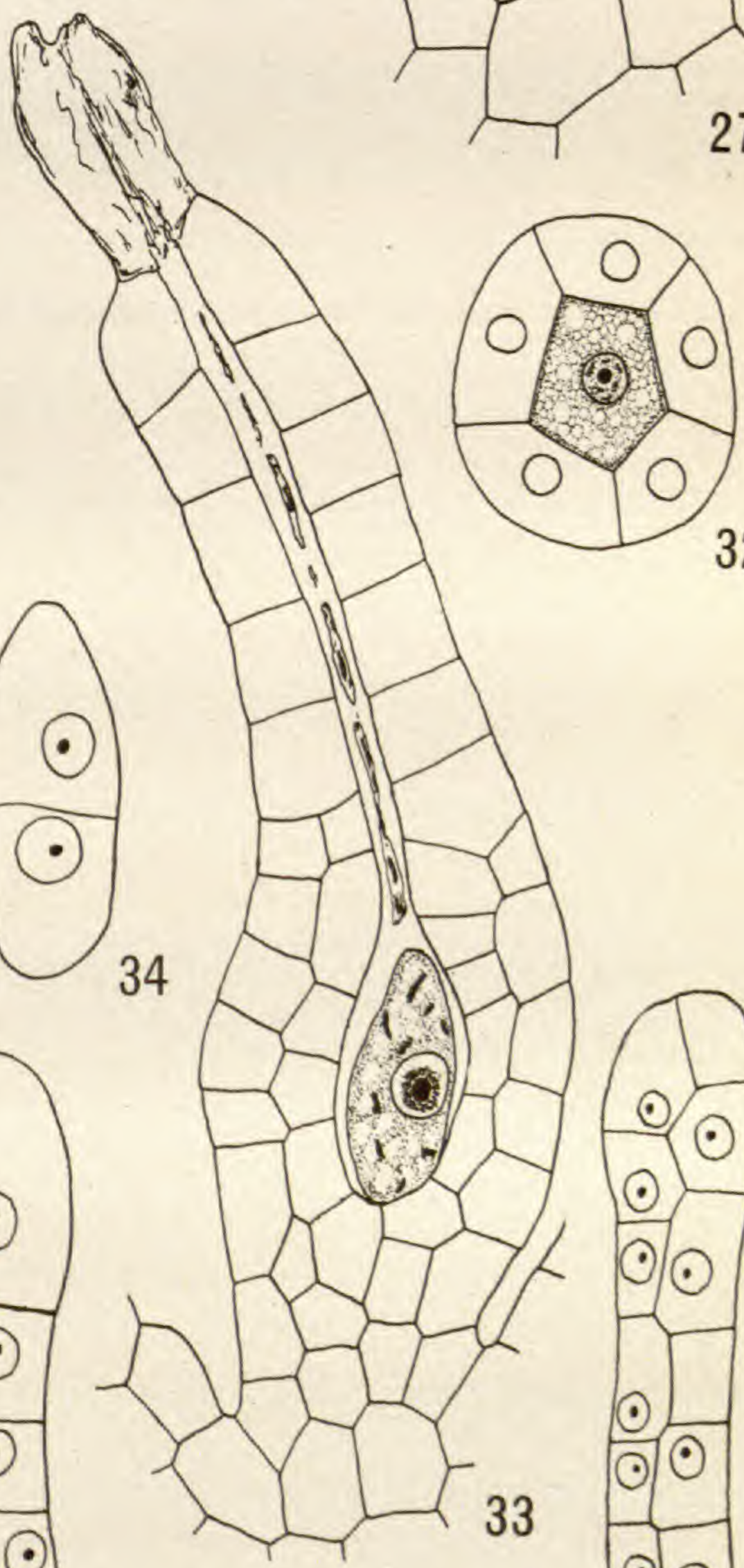
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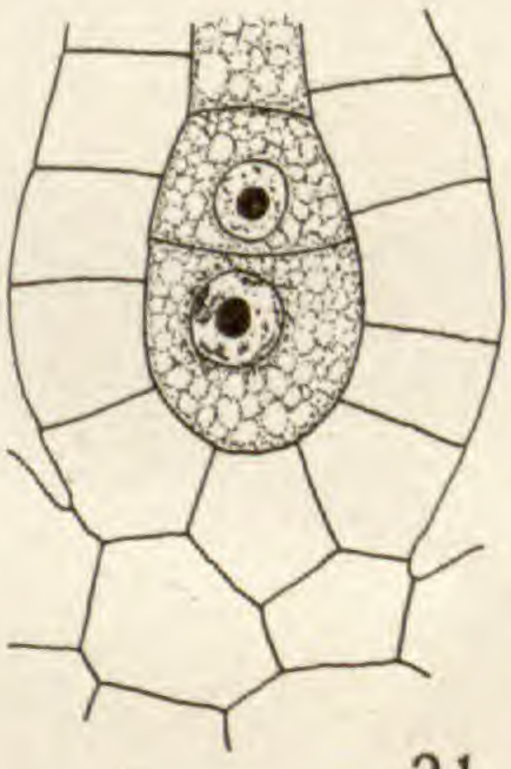
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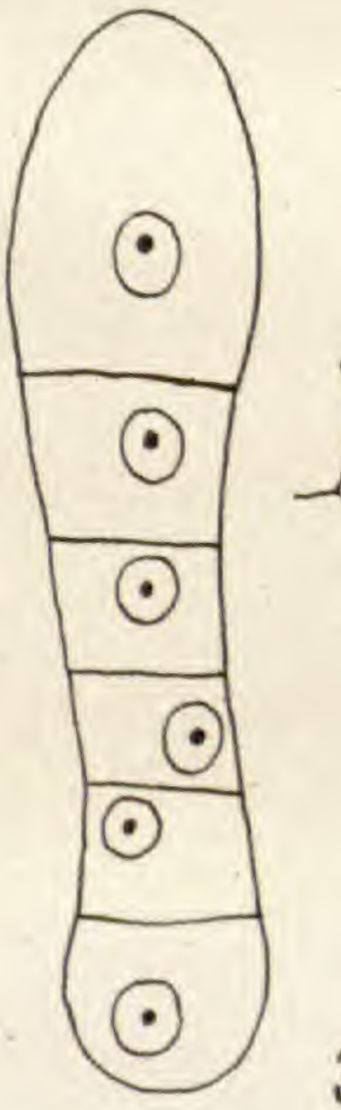
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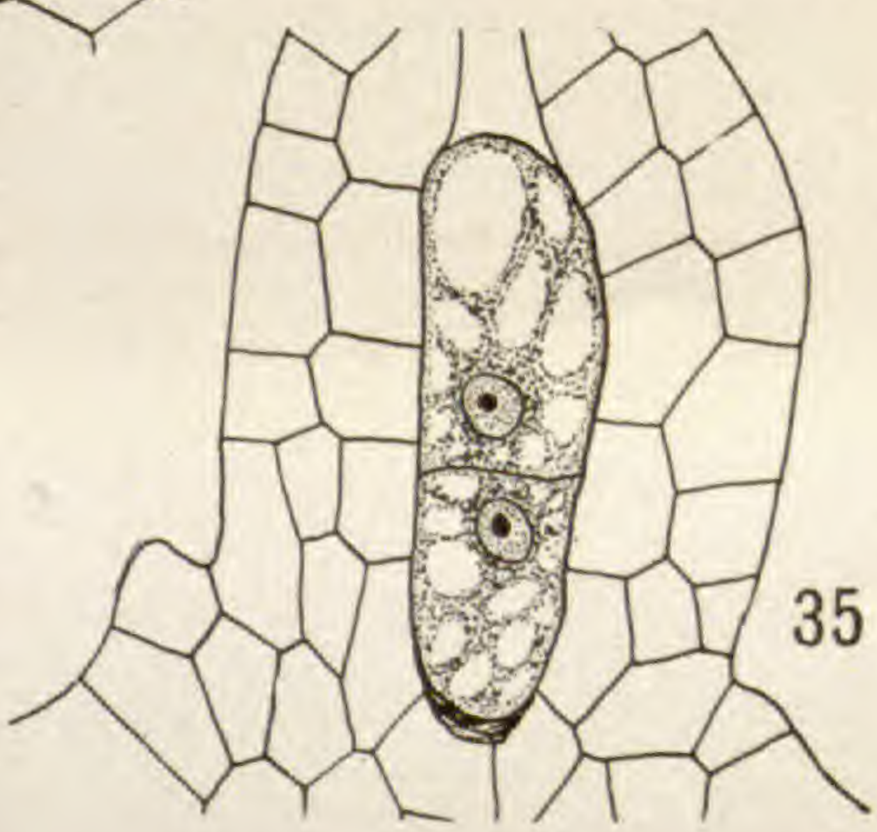
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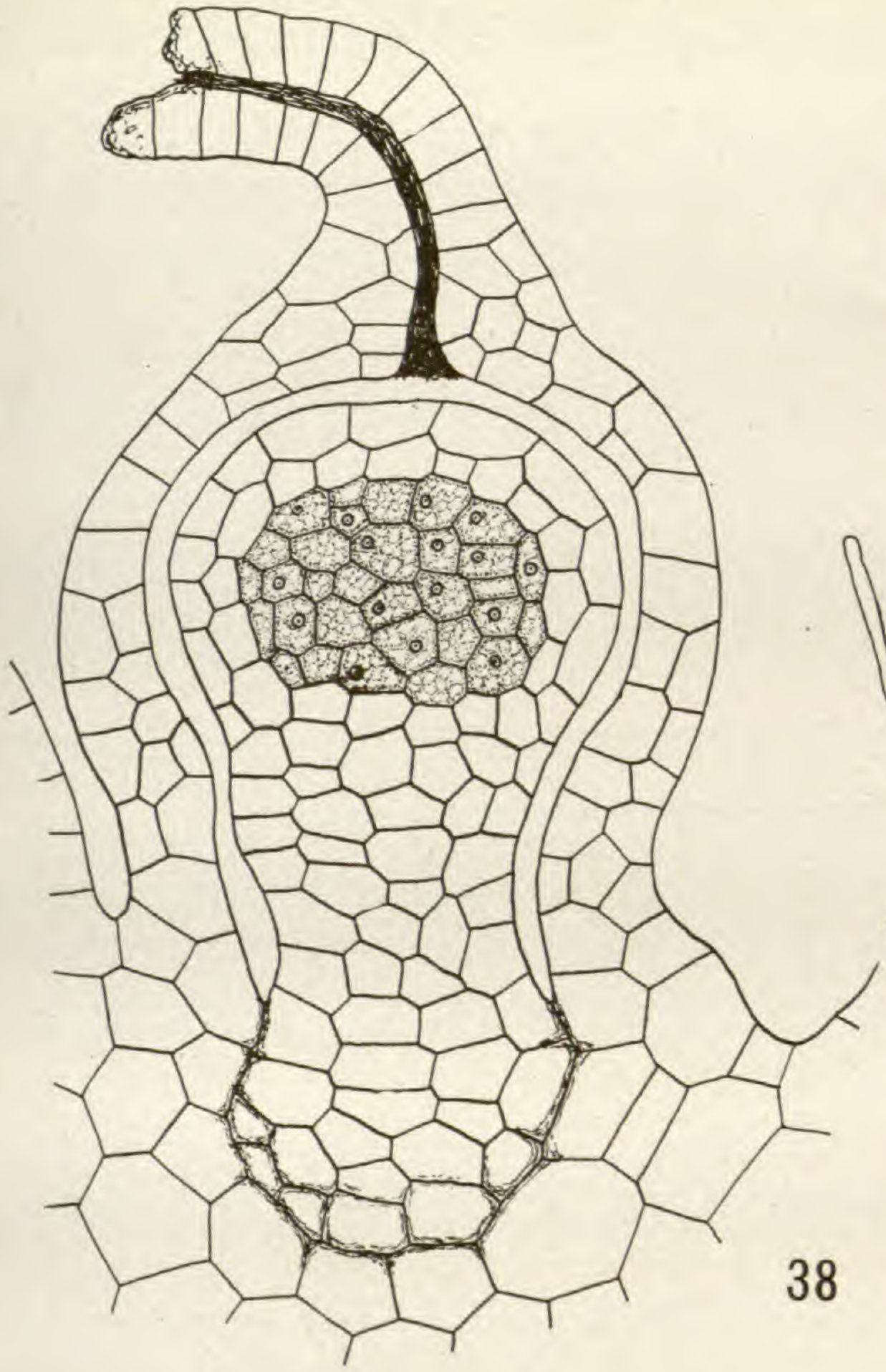
HAUPT on FOSSOMBRONIA



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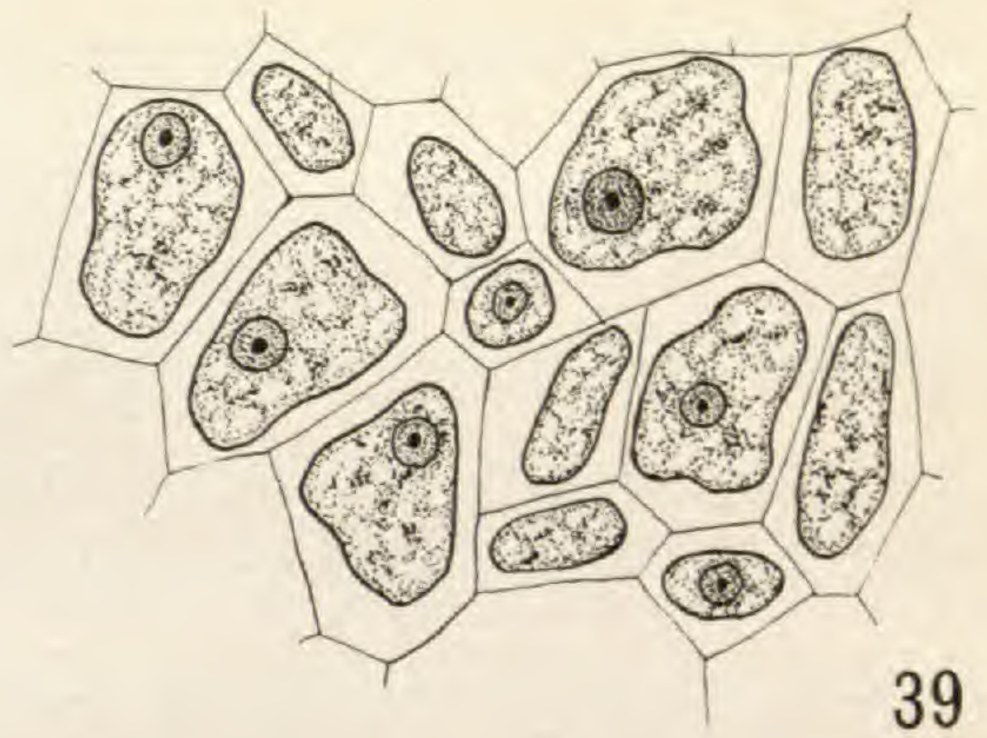

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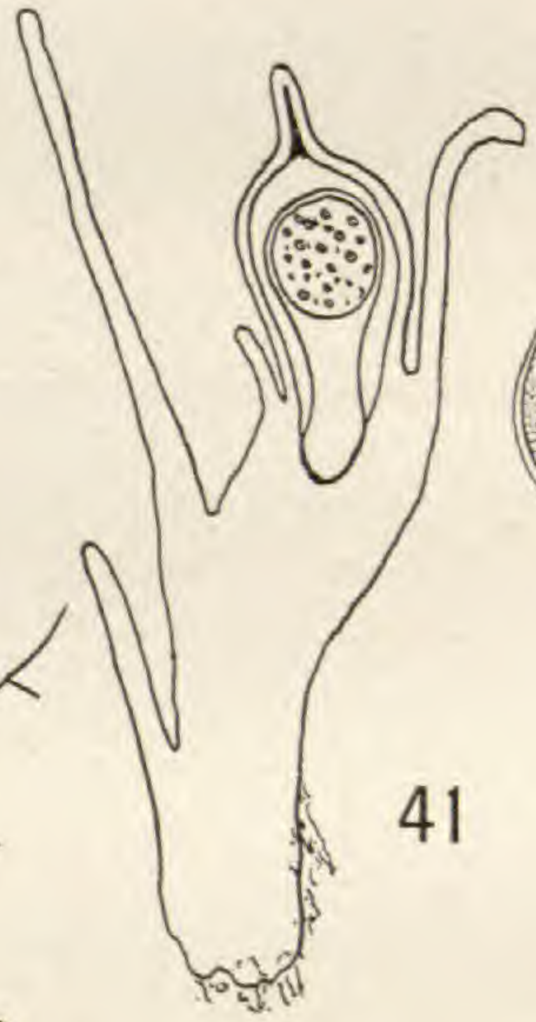

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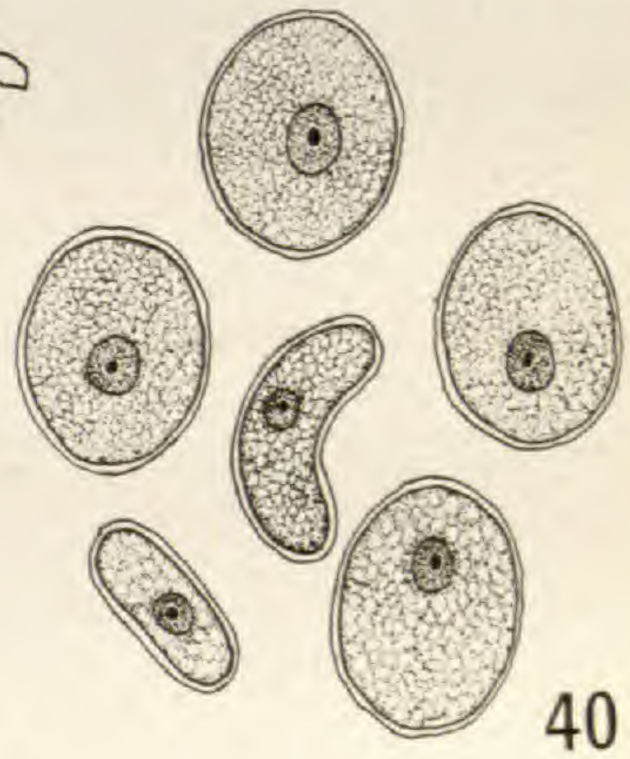

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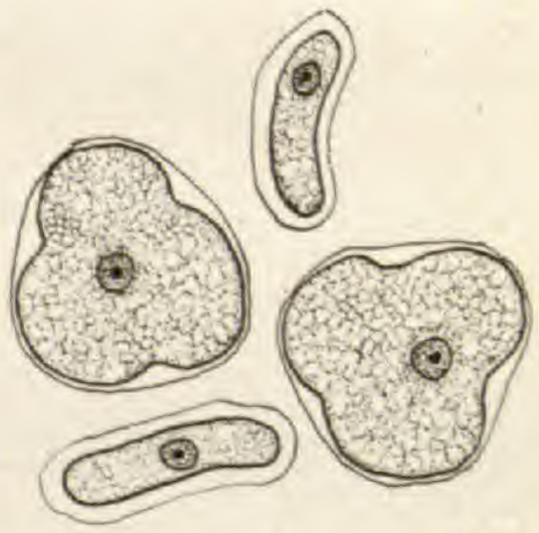

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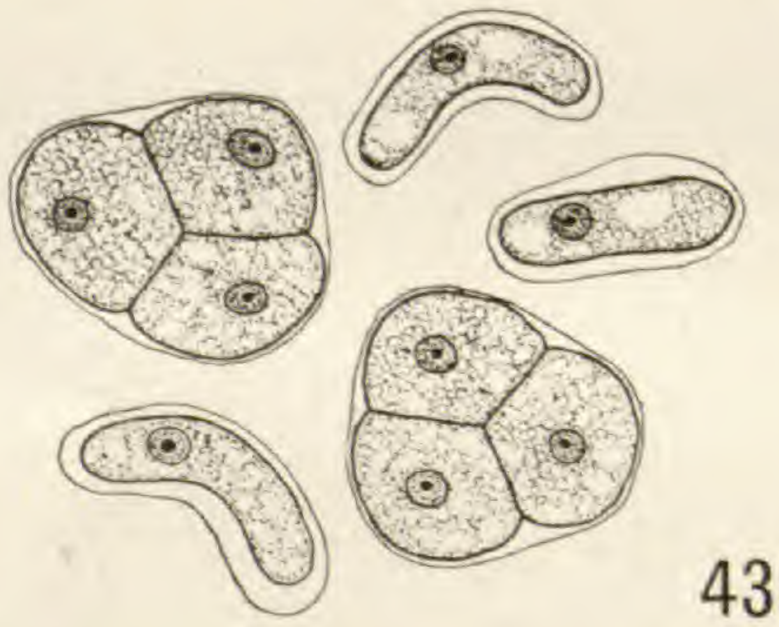

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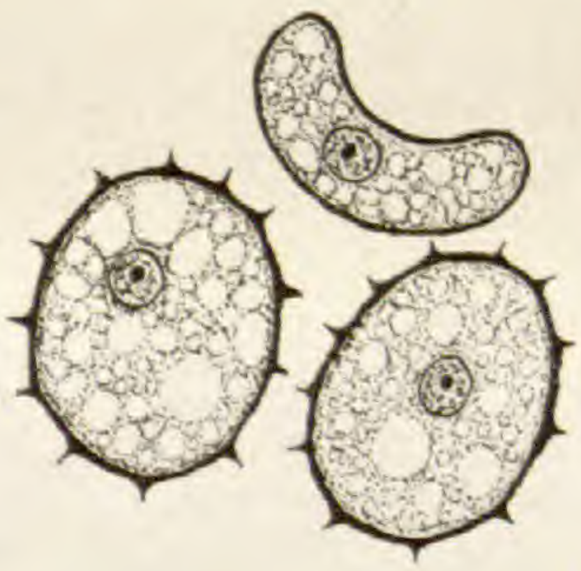

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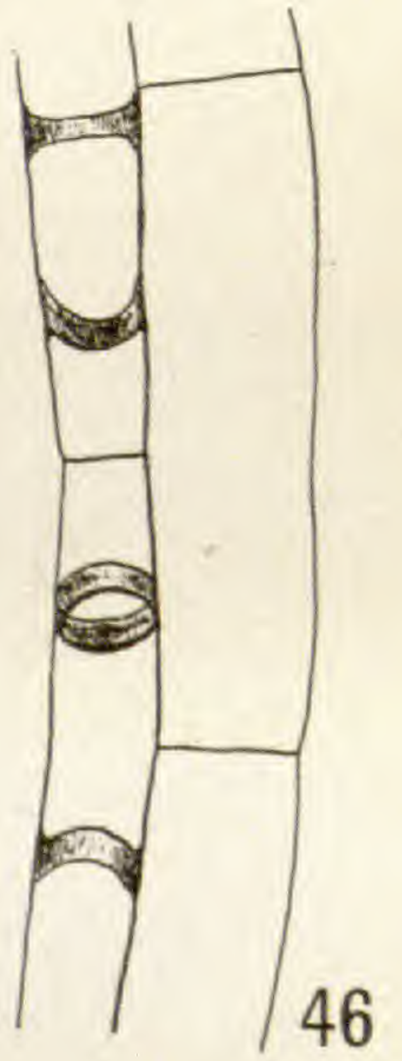

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HAUPT on FOSSOMBRONIA

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.

RESIDUAL EFFECTS OF CARBON DIOXIDE GAS ADDITIONS TO SOIL ON ROOTS OF LACTUCA SATIVA[1]

H. A. NOYES AND J. H. WEGHORST

(WITH FIVE FIGURES)

Variations in the development of roots of plants, when carbon dioxide gas is added subterraneously, have been described and reported in a previous paper.[2] The plants subjected to the carbon dioxide gas treatments were *Capsicum annuum abreviatum*, *Lactuca sativa*, *Raphanus sativus*, and *Phaseolus vulgaris*. The last three species were grown in the same soil, with fertilizer and manure treatments in addition to the check treatment already reported upon.

The treatment of the soil in the pots subsequent to the removal of the *Phaseolus vulgaris* plants in June 1917 was as follows. The soil in each pot was emptied into a large pan, thoroughly mixed, and returned to the pot. The water content was brought up to optimum, and one seedling of *Lycopersicum esculentum* placed in each pot. *L. esculentum* is considered a heavy potash feeder, and the plants were grown without carbon dioxide gas treatments in an endeavor to ascertain through plant growth the plant food made available by the previous gas treatments. The *L. esculentum* plants were harvested in November (5 months later), and the pots kept at near optimum moisture content until February 1, when they were again set to *Lactuca sativa*. The object of this test was to discover whether, on the addition of available nitrogen (in which the soil was lacking), more mineral plant food, made available by the carbon dioxide treatments of the previous spring, could be utilized by the growing plants. The moisture content of the soil in the pots was maintained at optimum by weighing and adding distilled water. Available nitrogen in the form of ammonium nitrate in quantities equivalent to 50 pounds of sodium nitrate per 2,000,000 pounds of soil was applied (with the distilled water added)

[1] Contribution from Purdue University Agricultural Experiment Station, La Fayette, Indiana.

[2] BOT. GAZ. 66:364. 1918.

on four dates, February 1, 9, 23, and March 27. The plants were harvested April 15 and the roots removed April 20, 1918.

The roots of the plants grown in the pots that had received the carbon dioxide gas applications the previous year had the malformations attributed to carbon dioxide in the previous paper. Where the soil had never been subjected to carbon dioxide treatments, the roots were well spread and extended considerably into the soil. Where carbon dioxide had been applied, the roots were shorter, spread out horizontally just beneath (0″ to 2″) the surface

FIG. 1.—Roots from unfertilized soil: left to right carbon dioxide treatments of soil were 0, 8, and 24 hours per day.

of the soil, and had tap roots that were abnormally short, crooked, and branching. The data with the fertilizer treatments are given in table I. The results show that something was left in the soil, due to carbon dioxide gas additions to the soil the previous year, which both shortened the tap roots and the distance below the crown at which the roots curved or split up into smaller roots. The residual effects of the gas were greater for the continuous than the intermittent treatments. The roots of the plants where the 24-hour treatments of carbon dioxide has been given were more affected under the manure than the fertilizer treatments.

FIG. 2.—Roots from soil fertilized with 5 tons of manure: left to right carbon dioxide treatments of soil were 0, 8, and 24 hours per day.

FIG. 3.—Roots from soil fertilized with single application of complete fertilizer: left to right carbon dioxide treatments of soil were 0, 8, and 24 hours per day.

FIG. 4.—Roots from soil fertilized with 10 tons of manure: left to right carbon dioxide treatments of soil were 0, 8, and 24 hours per day.

FIG. 5.—Roots from soil fertilized with double application of complete fertilizer: left to right carbon dioxide treatments of soil were 0, 8, and 24 hours per day.

The root of each set of three that had the best tap root was photographed, and is shown in figs. 1–5. The left hand root in each figure was grown in soil that did not receive carbon dioxide treatment; the middle one shows the residual effects of the 8 hours; and the right hand one shows the effects of 24 hours of gas treatments. With no gas treatment the roots of plants grown in manure tend to resemble those in which carbon dioxide gas was applied to the soil. This is confirmation of the statement made in the previous paper, namely, that "the results obtained in these experiments lead to the belief that the carbon dioxide content of garden soils is some-

TABLE I

RESIDUAL EFFECTS OF CARBON DIOXIDE GAS ADDITIONS TO SOIL ON DEVELOPMENT OF TAP ROOTS OF *Lactuca sativa*

PREVIOUS FERTILIZER TREATMENTS*	NO CARBON DIOXIDE GAS TREATMENT		8 HOURS' CARBON DIOXIDE TREATMENT DAILY		24 HOURS' CARBON DIOXIDE TREATMENT DAILY		FIG. NO.
	Length (in inches)	Distance to first curve (in inches)	Length (in inches)	Distance to first curve (in inches)	Length (in inches)	Distance to first curve (in inches)	
Nothing	5.2†	3.0†	3.7	1.3	2.8	0.9	1
Five tons dry manure‡	.05	5.0	4.7	1.6	4.5	1.4	2
Complete fertilizer (single application)§	4.9	2.3	4.5	1.7	4.2	0.9	3
Ten tons dry manure	4.4	3.4	3.9	2.0	1.9	1.3	4
Complete fertilizer (double application)	4.0	1.7	3.2	1.9	3.0	0.9	5
Average	4.7	3.1	4.0	1.7	3.3	1.1	.....

* In addition nitrogen was applied in ammonium nitrate on four dates at rate equivalent to 50 pounds sodium nitrate per 2,000,000 pounds soil.

† All figures are the average for three plants. ‡ Application per 2,000,000 pounds of soil.

§ Made from dried blood, dicalcium phosphate, and potassium chloride containing equal nitrogen, phosphorus, and potassium; nitrogen equal to one-third that in the 5 tons of dry manure.

times detrimental to the root development of some of the plants growing in the garden."

These residual effects of carbon dioxide additions to soil obtained over 9 months after the treatments were discontinued were unexpected, as the soil had been removed from the pots and mixed, and all water lost by evaporation added subterraneously. The explanation is not easy. The data are reported as a contribution to the knowledge of root growth, and it is hoped that it may help some workers in explaining odd tropic phenomena or throw some light on what is known as "soil toxicity."

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LEAF-BASE PHYLLODES AMONG THE LILIACEAE[1]

AGNES ARBER

(WITH FOUR FIGURES)

In a recent paper (1) the writer advocated the view that leaves of monocotyledons have no true laminae, but are either equivalent to petioles+leaf-bases, or are still further reduced until they reach the point of representing leaf-bases alone. In the paper cited, attention was mainly concentrated upon petiolar phyllodes, but in the present article it is proposed to review certain leaves among the Liliaceae which seem to be of leaf-base or leaf-sheath nature, and to consider the evidence upon which this interpretation is based.

There are a number of leaves among different tribes of the Liliaceae whose external appearance and general structure may well be taken to suggest a leaf-base origin. They show no differentiation into sheath and limb; they are parallel veined and furnished with a single series of normally orientated bundles. As examples *Hemerocallis*, *Tulipa*, and *Scilla* may be cited. That a view which presupposes a considerable power of development on the part of the leaf-sheath is not necessarily too extreme, is indicated by the fact that in some monocotyledons, in which there is a differentiation into sheath and limb, the sheaths may attain remarkable dimensions. For instance, the sheath of *Typha* may be half a meter long (3). Again, DOMIN'S (2) researches among the Umbelliferae have revealed a case in which all the foliage leaves are undoubtedly of leaf-base nature, namely, *Oreomyrrhis linearis* Hemsley. The linear leaves of this species, which bear a general resemblance to those of monocotyledons, terminate in a small rudiment apparently representing the blade.

There is not, in fact, any a priori difficulty in the way of interpreting the leaves of *Tulipa*, etc., as leaf-base phyllodes. We may now consider what positive evidence can be adduced in favor of this theory.

[1] This paper represents part of the work carried out during the tenure of a Keddey Fletcher-Warr Studentship of the University of London, and with the aid of a grant from the Dixon Fund of the University of London.

Ontogenetic evidence

Hemerocallis fulva L.—An apical bud of this plant was dissected on March 1. Neither in a leaf about 1 mm. long viewed under the simple microscope, nor in younger leaves examined with the compound microscope, could any distinction be discerned between the "leaf-sheath" and the rest of the leaf. The leaf is open to the extreme base, so that no closed sheath is formed.

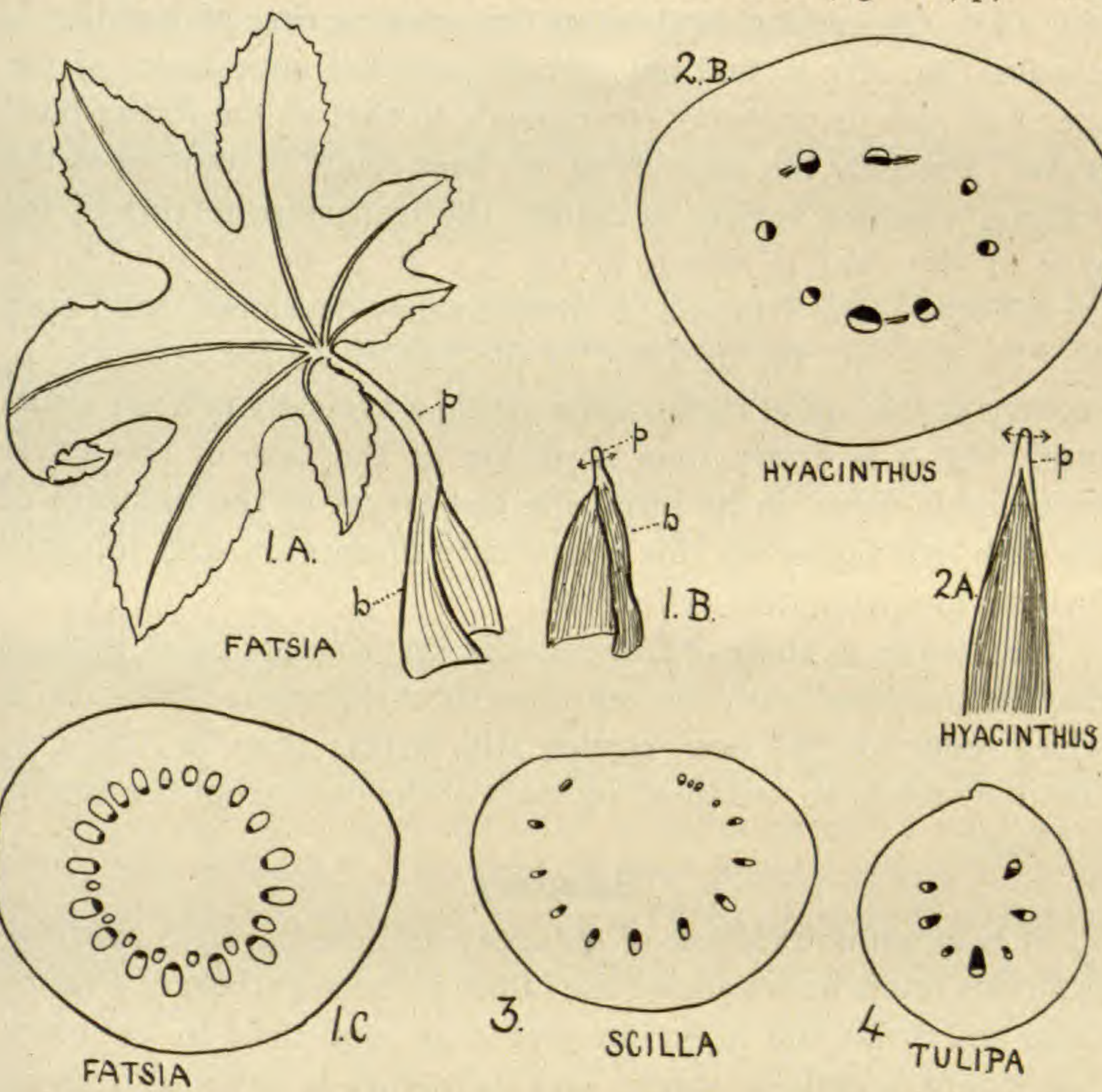
Scilla hispanica Mill.—The young foliage leaves for the current year were examined on March 1. All the leaves, down to the youngest, were found to be similar structures, in which the hooding of the apex was a relatively more conspicuous feature than in the older leaves. In the mature leaf, the sheath is seen to be closed for a very short distance at the base.

The conclusion to be drawn from the development of the leaves of these two species seems to be that in the case of *Hemerocallis* there is no evidence from the ontogeny of the existence in the leaf of any region except the leaf-base or leaf-sheath; in *Scilla* the main part of the leaf seems also to be of leaf-base nature, although the apical region of the hooded tip may possibly bear another interpretation, to which reference will be made later.

Evidence of comparative morphology

In order to test the interpretation here suggested, which explains the leaves of *Tulipa*, etc., as essentially leaf-base members, a search was made for some dicotyledon possessing both leaves with a well differentiated leaf-base, petiole, and lamina, and also reduced leaves which could be closely compared with those of the monocotyledons in question. Such a plant was found in *Fatsia japonica* Decne., of the Araliaceae, often cultivated under the name of *Aralia*. The normal foliage leaves of this plant are shown in fig. 1*A*. There is a well marked sheathing leaf-base (*b*), a petiole (*p*), and a palmate lamina. In addition, there are transitional leaf forms with reduced blades, culminating in bladeless bud-scales (fig. 1*B*). These are of the same nature as the leaf-base of the normal leaf, although they are thinner in texture, and the parallel veining is more obvious. The most interesting feature, however, is that the apical region of the bud-scale, which is developed in

varying degrees, is solid and approximately cylindrical, and may be interpreted as the rudiment of the leaf-stalk (fig. 1*B*, *p*). The



FIGS. 1–4.—Fig. 1, *Fatsia japonica* Decne.: *A*, small normal foliage leaf; *b*, leaf-base; *p*, petiole; *B*, bud-scale; *b*, leaf-base; *p*, rudiment of petiole; *C*, transverse section of apex of bud-scale at position marked with arrow in *B*; *A* and *B*, half natural size; *C*, ×23; fig. 2, *Hyacinthus* (garden var.): *A*, apex of leaf (half natural size); *B*, transverse section through apex of leaf shown in *A*, at level of arrow; ×23; fig. 3, *Scilla* (garden var.): transverse section through apex of leaf which was flat and dorsiventral except at tip; ×14; fig. 4, *Tulipa sylvestris:* transverse section through apex of leaf which was flat and dorsiventral except at tip; form on upper side shows first indication of opening into main flat part of leaf; ×23.

transverse section of this region shows a slightly dorsiventral ring of bundles (fig. 1*C*), so that the anatomy is distinctly petiolar.

When we turn to the monocotyledonous leaves which we wish to interpret, we find that in certain of them there is an apical structure which closely parallels the petiole rudiment of the

bud-scales of *Fatsia*. In the garden hyacinth, for instance, the leaves may often be found to terminate in a short, solid, cylindrical apex (fig. 2*A*). On cutting sections of this apex, a ring of bundles is revealed (fig. 2*B*), so that not only the external appearance of the apex but also its anatomy corresponds to that of the *Fatsia* bud-scales. Precisely the same thing has been found in another of the Scilleae, a garden variety of *Scilla;* the transverse section of the apex of this leaf is shown in fig. 3. In a second subtribe of the Lilioideae, the Tulipeae, a conspicuously developed, solid apex may be observed, for instance, in the leaf of *Tulipa sylvestris* L. Sections of this apical region again reveal a typically petiolar structure. Fig. 4 is drawn from a section at the base of the apical region, and shows, in its form, the last traces of the influence of the limb, but higher up this irregularity disappears, and the apex becomes approximately cylindrical.

Such leaves as those of *Hemerocallis*, on the other hand, perhaps may be compared with the countless dicotyledonous bud-scales in which reduction has been carried still farther than in *Fatsia*, so that they retain no vestige of any part of the leaf except the sheathing base.

Summary

It is shown on evidence of ontogeny and comparative morphology that certain leaves among the Liliaceae, such as those of *Hemerocallis* and *Scilla*, are to be interpreted as equivalent to leaf-bases. The lamina is entirely absent, and the petiole is either also absent or is present in an extremely reduced form. The solid, approximately cylindrical apices in which the leaves of *Hyacinthus*, *Tulipa*, etc., sometimes terminate, are held to represent the last rudimentary phase of the vanishing petiole.

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3. ———., Morphologische und phylogenetische Studien über die Stipularbildungen. Ann. Jard. Bot. Buitenzorg **24**:117–326. *pls. 23–33.* 1911.

DEVELOPMENT OF THE GEOGLOSSACEAE[1]

G. H. DUFF

Although a number of investigators have contributed developmental studies on the Ascomycetes and very substantial progress has been made, our knowledge of the ontogeny of the higher forms of these fungi is still far from complete. In consequence, our present systems of classification are full of gaps, and our conceptions of the affinities of these plants are often contradictory or mere guesses. For the elaboration of a satisfactory system of classification and for the consolidation of our ideas regarding relationships, it is requisite that the ontogeny of a much larger number of representative species be worked out.

This investigation has been confined to the Geoglossaceae. Observations have been made on practically complete stages of *Cudonia lutea*, *Spathularia velutipes*, and *Trichoglossum hirsutum*, and on some of the critical features of *Leotia*. Heretofore studies in this family have been restricted to three species of the genera *Leotia* and *Mitrula*.[2] The chief interest centers around *Cudonia lutea* and *Spathularia velutipes* because of the remarkable ascogonia possessed by these plants, and because of the conspicuous veils which render obvious to the naked eye their angiocarpous nature, and which have long stood in opposition to the distinction by which SCHROETER[3] separates the Helvellineae from the Pezizineae.

The youngest stage of *Cudonia lutea* which has come under observation is in the form of a minute cushion of interwoven threads measuring but 84 μ in height. At the center of this loose assemblage of threads may be seen a small but definite group of hyphae which are rendered conspicuous by their size and staining qualities. These are not ascogonia, as might at first be

[1] Preliminary communication.

[2] DITTRICH, G., Zur Entwickelungsgeschichte der Helvellineen. Cohn's Beiträge 8:1. 1918.

BROWN, W. H., The developement of the ascocarp of *Leotia*. BOT. GAZ. 50:443–459. 1910.

[3] SCHROETER, J., In ENGLER and PRANTL, Die natürlichen Pflanzenfamilien.

supposed, but are the precursors of coiling procarps which arise from them at a later stage, in a manner to be described.

So far as the writer is aware, such a sequence of structures has not elsewhere been reported for any species of the Ascomycetes proper. Among the lichens, however, a similar condition has been recorded. In a paper dealing with the ontogeny of the ascocarp of several forms of lichens, NIENBURG[4] figures and describes bodies which are differentiated early in the process of development, and which at a later stage give rise to "carpogones." These bodies are designated "generativen hyphen" by this author. Following his usage, the term "generative hyphae" will be employed in reference to the threads here described and to their immediate proliferations.

The next developmental stage exhibits a distinct differentiation of vegetative tissues. There is now present a well organized outer covering, which completely envelops the looser tissues, and at the center the generative hyphae are more conspicuous than ever. By this time the generative hyphae have proliferated to a slight extent, and appear as a somewhat larger and more compact group of threads with an extraordinary affinity for stains. As growth proceeds the outer tissue expands, remaining in its peripheral position as a true veil. Its persistence and growth are not functions solely of the tissues that lie beneath it, but of itself as well. By its own growth it is able to keep pace for a considerable time with the rapid enlargement of the cap, a fact that is true even of that portion which is eventually separated from its connections by the developing hymenium. This growth, in contrast with mere stretching, results in a marked increase in the thickness of the veil, measurements showing that the earliest envelopes average about 20 μ, while at maturity they approach 70 μ in thickness. The veil ruptures over the hymenium only, and there only after the latter is well matured.

By upward growth and by the appearance of a mass of what may be termed parenchymatous tissue at the base of the young fruit, the generative hyphae are forced to assume a subapical

[4] NIENBURG, W., Beiträge zur Entwickelungsgeschichte einiger Flechtenapothecien. *Flora* **98**: 1907–1908.

position. This position is retained until they give rise to the procarps. At this time the height of the fruit body is about 2 mm., and the cap has been well differentiated from the stem. At such a stage the generative hyphae largely fill the upper portions of the cap, and the procarps arise as branches from these hyphae. The procarps are numerous, coiling, and deeply staining structures, scattered irregularly throughout the cap. These coils are continued upward by what appear to be "typical" multiseptate trichogynes, which penetrate the envelope, projecting into the air for a short distance. Spermogonia and spermatia are entirely lacking, and it is not thought that the trichogynes are functional organs.

Despite the great difficulty of staining differentially both the generative hyphae and the procarps, owing to the remarkable affinity for stains exhibited by these structures, there is sufficient evidence to show that the cells of the procarp, including those of the trichogyne, are originally uninucleate. Later the ascogonial cells become multinucleate, the nuclei being small and paired; and ascogenous hyphae arise from them into which these nuclei probably pass.

It is important to note that up to this time there has been no sign of a hymenium. The fruiting surface now makes its first appearance in the form of paraphyses immediately beneath the veil. Before the paraphyses have attained their full development the ascogenous hyphae, that meanwhile have taken their origin from the procarps in close proximity, and have rapidly proliferated and gone through various evolutions of hook formation, begin to organize asci. This young hymenium is inclosed by the veil, and remains so until many of the asci are mature and spore discharge is ready to commence. The nuclear phenomena preceding spore formation are typical in their chief features.

The developmental history of *Spathularia velutipes* follows a course not unlike that of *Cudonia lutea*. The youngest fruits of this species that have been examined are somewhat larger than the youngest species of *Cudonia*, being in the neighborhood of 0.5 mm. in height. At this stage the young *Spathularia* is covered with an envelope, but the inner tissues are quite undifferentiated, and there are as yet no signs of any structures resembling the generative

hyphae of *Cudonia*. In the next stage of the series, however, threads resembling generative hyphae are visible, and they have already taken up their position just behind the apex of the somewhat cone-shaped ascocarp. The envelope here is worthy of some remark, inasmuch as it is easily differentiable by staining into two parts, an outer and an inner. The inner tissue is capable of growth and is responsible for the persistence of the veil in *Spathularia*, and for the continued production of the outer tissue which becomes split by the growth of the fruit body into adhering masses of cells which are responsible for the velvety appearance from which the species derives its name. Measurements of the thickness of the envelope in the youngest and in mature specimens here also indicate the extent of this growth, and show the veil to be capable of doubling in thickness, increasing from about 25 to 50 μ. This is but a rough and inadequate index, however, since the outer tissue may be considerably worn away.

Procarps of a very much reduced nature are produced in *Spathularia velutipes*. These appear even later than those of *Cudonia*, arising after the formation of paraphyses. They are more variable in size and shape, and do not possess trichogynes. They are responsible for the initiation of the paired condition of the nuclei, and ascogenous hyphae may be seen arising from them. The entire ascogonial system in *Spathularia* is just as refractory with respect to stains as that of *Cudonia*, and nuclear details, consequently, are very difficult to obtain. In all other respects *Cudonia* and *Spathularia* resemble one another closely.

Examination of a complete series from a very young stage to maturity has shown that *Trichoglossum hirsutum* is not possessed of a veil at any time in the history of the development of its fruit body. The long setae that characterize the ascocarp of this species, however, are present from the very first. This condition is noteworthy, inasmuch as it is very similar to that which FITZPATRICK[5] has described for *Rhizina undulata*. In these two species we have the only members of the Helvellineae whose developmental history has as yet been described, for which the presence

[5] FITZPATRICK, H. M., The development of the ascocarp of *Rhizina undulata* Fr. BOT. GAZ. **63**:282–296. 1917.

of a veil at some stage of their development has not been claimed, and each is provided with these remarkable setae. In matters of sexuality *Trichoglossum* appears to be still more reduced than *Spathularia*. Ascogenous hyphae arise from threads which are little if at all differentiated from the vegetative hyphae.

Although DITTRICH (*loc. cit.*) claims for *Leotia lubrica* the possession of a veil in its younger stages, BROWN (*loc. cit.*), in his more recent paper on this species, makes no mention of the occurrence of any such structure, and apparently has observed none. A tissue overlying the hymenium has been observed by the writer in a fairly well advanced specimen during the course of a cursory examination of this form. Younger stages which show this covering have not been found, however, so that considerable uncertainty obtains with regard to the identity of this tissue with that figured by DITTRICH.

A point of very great interest in this investigation is the close resemblance of the conditions described for these Geoglossaceae to those which NIENBURG attributes to the *Cladonia*-like lichens *Icmadophila*, *Sphyridium*, and *Baeomyces*. The occurrence in these lichens of generative hyphae which later give rise to carpogonia has already been mentioned. These carpogonia are "typical" coils with trichogynes in *Icmadophila;* but they are progressively more degenerate in *Sphyridium* and *Baeomyces*, in the last of which NIENBURG was unable to distinguish their presence with certainty. Further points of similarity include the occurrence of an envelope in the early stages, and the methods of ascus formation. This remarkable parallelism evidently represents a relationship. Although a general relationship between the Ascolichens and other ascomycete groups, such as the Discomycetes and Pyrenomycetes, has long been recognized, and although some lichenologists have advocated and attempted the distribution of the lichen genera among those of other Ascomycetes, a fundamental basis of relationship between the discolichens and the order Helvellineae has been wanting. This basis is supplied here and consists of a close similarity in developmental history, particularly with regard to the veil and to the manner and time of appearance, number, position, and condition of procarps. As our knowledge of these

forms increases, the extent of this relationship will unquestionably be more clearly shown.

A detailed illustrated account of this work is to be published in the near future.

The writer desires to acknowledge his indebtedness to Professor J. H. FAULL, of the University of Toronto, under whose guidance this investigation has been prosecuted, and to express his thanks for valued direction and criticism.

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BRIEFER ARTICLES

THE CINCHONA STATION

The lease of the Cinchona Station by the Smithsonian Institution on behalf of a group of contributing American botanists was interrupted by conditions existing during the war. It has now been resumed, and the laboratory will be available for American botanists during the coming year.

This tropical laboratory, in a well kept botanical garden containing many exotic trees, shrubs, vines, and herbaceous perennials from all quarters of the earth, is located at 5000 ft. elevation, on the southern slope of the rugged Blue Mountains of Jamaica, within half an hour's walk of an undisturbed montane rain forest.

The dry ridges and sunny valleys of the south side of the Blue Mountains offer many types of peculiar ferns, epiphytic bromeliads, grasses, mistletoes, and lianes. In the rain forest of the north side are to be found many species of liverworts, mosses, and ferns, the latter ranging from the very diminutive epiphytic species of *Polypodium*, only an inch or two in height, to the scrambling species of *Pteridium*, *Gleichenia*, or climbing *Lomaria* of many yards in length, and the great tree ferns, 40 ft. in height. There are also many interesting native species of trees, shrubs, and vines which together make parts of the forest a practically impenetrable jungle. There are great stretches of the northern slopes of the Blue Mountains, within a day's walk of Cinchona, that have never been explored by the botanist, not even by the collector.

Botanists wishing to study plants of the lowlands or of the sea coast can make their headquarters in Kingston, and such workers have always had the use of the library, herbarium, and laboratory at Hope Gardens. These gardens also contain a fine collection of native and introduced tropical plants, offering much material for morphological and histological study. Cacti, agaves, and other xerophytic plants of the seacoast, and the algae of the coral reefs along the shore, afford still other types of vegetation of great ecological, developmental, and cytological interest. Castleton Garden, the third botanical garden of the island, has a very different climate from either Cinchona or Hope, for it is located in a hot steaming valley, 20 miles north of Kingston, where

cycads, screw pines, palms, orchids, figs, ebonies, the gorgeous *Amherstia*, and many other tropical trees grow luxuriantly.

All in all, Jamaica probably offers the botanist as great a variety of tropical conditions within a day's walk of Cinchona and a day's drive from Kingston as can be found anywhere in an area of this size. It is evident that the opportunities for the study of many kinds of botanical problems are abundant at Cinchona, Hope, and Castleton. In fact, there are many botanical problems of prime importance which can be studied only in such environments.[1]

Any American botanist wishing to work at Cinchona may be granted this privilege by the Cinchona committee, consisting of N. L. BRITTON, J. M. COULTER, and D. S. JOHNSON. Inquiries for this privilege and for information regarding the conditions under which it may be granted should be sent to the writer.—D. S. JOHNSON, *Johns Hopkins University, Baltimore, Md.*

CHROMOSOME NUMBER IN THE SEQUOIAS

For some years we have been concerned with cytological studies in the genus *Sequoia*. In particular a review of the evidence presented by LAWSON[2] on the life history of *S. sempervirens* has been attempted. That considerable interest attaches to this genus is obvious, and certainly the information available in regard to the life history of *S. gigantea* is meager. The present note is intended primarily to call attention to certain points which have been indicated in our preliminary studies.

LAWSON reports that, in his material collected at Stanford University, California, the pollen grains are formed during the second or third week of December, and that the pollen is shed during the first week of January.[3] In our experience, extending over some three years, the pollen is often mature in September and rarely is it found on the tree after November. Our observations have been made on trees of the same size growing in three different localities: Berkeley, Redwood Peak, and Mill Valley, California. There is great variation in the time of pollen shedding. Two trees standing side by side may show a difference of two weeks to a month in the occurrence of this phenome-

[1] For further details see Science 43:917. 1916, and Popular Science Monthly, January, 1915.

[2] LAWSON, A. A., The gametophytes, archegonia, fertilization, and embryo of *Sequoia sempervirens*. Ann. Botany 18:1–28. 1904.

[3] SHAW, W. R., Contribution to the life history of *Sequoia sempervirens*. BOT. GAZ. 21:332–339. 1896.

non, and in any two consecutive seasons an individual tree may shed pollen on dates separated by a corresponding interval of time. In the same way it has been found impossible to predict with any degree of accuracy the time of occurrence of any of the significant stages in maturation, and this fact has rendered more difficult the determination of chromosome number in *S. sempervirens*. Numerous efforts so far have failed to discover the reduction divisions in the microspore mother cells.

As to chromosome number in *S. sempervirens*, LAWSON remarks that "as near as could be estimated, there are 16 chromosomes in the gametophyte and 32 in the sporophyte." In recent tabulations of chromosome numbers in plant species, 45 gymnosperms are listed. All but 12 of these have *x* 12, and *2x* 24, and of these 12 (*x* 16 and *2x* 32) a number are listed as doubtful. On this basis perhaps there might be legitimate ground to question LAWSON's count. In sections of root tips of *S. sempervirens* we have made counts which only in rare instances confirm LAWSON's report. The difficulties are great in such material, however. In corresponding and more favorable material of *S. gigantea*, we have uniformly counted from 21 to 24 chromosomes, but never a greater number.

With these facts in mind, the following possibilities present themselves. First, if LAWSON's count is correct for *S. sempervirens* and if our count is correct for *S. gigantea*, the two species have different chromosome numbers. Second, if our suspicion of LAWSON's count in *S. sempervirens* is valid and if our count in *S. gigantea* is correct, both species have *x* 12 and *2x* 24. The third possibility involves an inaccuracy in our count of *S. gigantea* and chromosome numbers 16 and 32 for both species. In our opinion the second possibility is the only one which merits serious consideration. It seems worth while, however, to present the whole situation, since the other possibilities cannot wholly be left out of account with the data at hand. Further studies will involve an investigation of the life history of *S. gigantea* and the obtaining of a final conclusion as to chromosome number in *S. sempervirens*.—T. H. GOODSPEED and M. P. CRANE, *University of California*.

CURRENT LITERATURE

BOOK REVIEWS

Ecology of tide lands

There is no place more suitable for the study of dynamic ecology than in areas swept over by the tides, and there is no one better able to write on the problems of such areas than Professor OLIVER.[1] For years he and his students have attacked seashore problems, first on the coast of Brittany, and more recently on the coast of Norfolk. The Bouche d'Erquy and Blakeney are household words to all students of shore ecology. The main results of OLIVER'S studies are now incorporated in book form, and, quite in the spirit of the time, he has become associated with an engineer, who presents the practical application of ecological principles to engineering problems along shore; the result is a masterpiece of applied ecology.

The first chapters deal with tide and current data, the tidal compartments of rivers, and the foreshore. That the problem is one of no mean importance is shown by the fact that in the British Isles there are 8000 miles of shore line and 11,000 miles of river front at high water; and there are 1250 square miles of area between tides. OLIVER'S greatest contribution is in chapters iv–vii, which deal with the function of vegetation, sand dunes and their fixation, and shingle beaches and their fixation. The fundamental importance of plants in the stabilization of shore lines has been inadequately realized by engineers, although sporadic and often ineffectual planting of sand dunes has been more or less indulged in for a century. A perusal of this work makes it clear that ecology must form a large part of the education of an engineer who really wishes to get at the foundations of shore problems. So far as dunes are concerned, Britain's problem is not as great as that of Gascony and other continental tracts. The most satisfactory plant for dune fixation is *Psamma* (*Ammophila*), although *Elymus arenarius*, *Carex arenaria*, and other species may also be used. Even lichens and mosses have a fixative value. The chief factor in dune fixation lies in the development of an effective foredune.

One of the striking features of British shores is the shingle beach, where cobblestones are piled up by vigorous wave movement. At Dungeness the shingle covers 10,000 acres. At Blakeney on the Norfolk coast the shingle is piled up to a height of 10 feet above high water, and at Chesil on the Atlantic shore, the height is 30 feet. Shingle is kept mobile (1) by wave impact and

[1] CAREY, A. E., and OLIVER, F. W., Tidal lands; a study of shore problems. 8vo. pp. 284. *pls. 29. figs. 54.* London: Blackie & Son. 1918.

throw, resulting in a talus or fan on the lee side, (2) by percolation, especially where there is large tidal difference, or (3) by stream scour on the lee side. *Suaeda fruticosa* is able to colonize upward growing shingle, quite as *Psamma* may colonize an upward growing dune; *Suaeda* is an especially good pioneer, because of its halophytic proclivities. Later stages, as shingle growth decreases, are characterized by mat plants such as *Silene maritima* and *Convolvulus Soldanella*. A plant of the latter increased in area within four years from 9 to 525 square feet.

An interesting chapter deals with the reclamation of salt marshes. It is OLIVER's view that a marsh would not fill alone by silting, by reason of alternate filling and cutting. Reclamation may be brought about naturally by coastal elevation or by the building up of a barrier dune, or it may be brought about by artificial agencies. A remarkably effective plant reclaimer of halophytic shores is *Spartina Townsendii*, a supposed natural hybrid of *S. stricta* and *S. alterniflora*. This species was first noted at Southampton in 1870, and now covers thousands of acres. In 1895 it appeared at Bayonne, on the Bay of Biscay. It is interesting to note that these two areas are the only ones known where the areas of the supposed parent species overlap. —H. C. COWLES.

NOTES FOR STUDENTS

Root systems.—Since the notable work of CANNON in 1911 on the roots of desert plants, nothing has contributed so much to our knowledge of subterranean plant organs as the recent publication by WEAVER,[2] in which he has described the root systems of some 140 species of shrubs and herbs from the prairies of Nebraska and Washington, the plains and sand hills of Colorado, and some gravel slide and forest communities of the Rocky Mountains of Colorado. For each of the habitats under investigation many data regarding such environmental conditions as rainfall, evaporation, and soil moisture are given. These data and the abundance of illustrative drawings and photographs of excavated root systems are among the most valuable features of the report.

In the Nebraska prairie there is a striking individuality in the root systems, and a grouping of the roots into more or less definite absorbing layers, thus reducing competition and permitting the growth of a larger number of species. The deeper rooted species comprise 55 per cent of the 33 species examined, and extend beyond a depth of 5 feet, some reaching as much as 20 feet below the surface, many of them having few or no absorbing roots in the first few feet of soil. The majority of the deeply rooted species are dicotyledons; but it is notable that the group also includes three dominant grasses, *Panicum virgatum*, *Andropogon furcatus*, and *Agropyron repens*. In contrast

[2] WEAVER, J. E., The ecological relations of roots. Carnegie Inst. Wash. Publ. 286. pp. vii+128. *pls.* 33. *figs.* 58. 1919.

with this group, all plants with roots confined to the upper 2 feet of the soil are grasses, and include such species as *Koeleria cristata*, *Stipa spartea*, *Elymus canadensis*, and *Distichlis spicata*.

Such root systems are to be related to the deep, mellow, loess soil with high water-holding capacity and moist subsoil. Here the data of WEAVER correspond well with those of ALWAY[3] for moisture conditions, although the former finds a much deeper root development than that assumed by the latter. In the upper 4 or 5 feet there is usually at midsummer a reduction of the water supply to a point below the wilting coefficient, these data corresponding with those of the reviewer for the grasslands of the Chicago region.[4]

The climatic conditions of the prairies of southeastern Washington are shown to be more severe than those of Nebraska, not only because of a smaller annual precipitation, but also because only one-third of this rainfall comes during the growing season. As a part of the response, the early flowering grasses predominate, and many of these, such as *Koeleria cristata*, *Poa Sandbergi*, and *Festuca ovina* have their roots confined to the upper 18 inches of soil. There remain, however, some grasses and many dicotyledons that are decidedly deep rooted.

Some data also are given for a "chaparral" community transitional from the prairie to the forest, and dominated by species of *Symphoricarpos*, *Rhus*, *Corylus*, and *Rosa*. The designation is unfortunate, for the best usage would limit the term "chaparral" to an evergreen scrub like that occurring on the Pacific Coast of California.

In comparison with the root systems of the prairies, those of the plains are characterized by a larger percentage of moderately deep rooted species, fewer very deeply rooted plants, and by a more extensive system of surface absorbing and wide spreading laterals. SHANTZ[5] reported that at Akron, Colorado, almost the entire root system of all the grasses is limited to the 18 surface inches. The conditions are evidently different near Colorado Springs, for there WEAVER reports one grass only, *Koeleria cristata*, with roots confined to the surface 2 feet. Grouping into layers is again evident; the most distinctive feature of the plains species, in addition to spreading laterals, is the moderate penetration of the deep rooted species. This is doubtless due, as indicated by both WEAVER and ALWAY (*loc. cit.*), to the comparative impenetrability of the extremely dry subsoil.

The sand hill community exhibits in a still more striking manner the development of a profusion of widely spreading laterals in the upper 2 or

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[3] ALWAY, F. J., *et al.*, Relation of minimum moisture content of subsoil of prairies to hygroscopic coefficient. BOT. GAZ. **67**:185–207. 1919.

[4] BOT. GAZ. **58**:193–234. 1914.

[5] SHANTZ, H. L., Natural vegetation as an indicator of the capabilities of land for crop production in the great plains area. U.S. Dept. Agric., Bur. Pl. Ind. Bull. 201. pp. 100. *pls. 6. figs. 23.* 1911.

3 feet of soil. This is true even of the deep rooted species, and is doubtless to be related to distribution of soil moisture. It is notable that MARKLE[6] found a similarly abundant development of even more superficial laterals in the very arid conditions in New Mexico. MARKLE also described and figured a considerable variety of systems, and found rather definite layers of penetration lessening competition for the scarce water supply.

In the succession from the gravel slide with coarse soil to the forest rich in humus, the Colorado Rocky Mountains afford an interesting series. WEAVER finds decidedly superficial systems, both in the very sparsely populated gravel and in the undergrowth of the forest, with the moisture distribution the controlling factor in each case. The intermediate half gravel slide, with its surface more than half occupied with plants, curiously enough has more deeply rooted plants than the associations preceding or succeeding it.

A comparison of species occurring in two or more different habitats shows that of 10 species examined, 7 exhibit changes in root habit in response to the changed environment, while 3 remain quite constant. Such studies of the response of root systems to environment have attracted the attention of other workers. WATERMAN[7] finds roots developing under dune conditions somewhat responsive to organic remains in the sand, although usually adhering rigidly to their specific inherited form. Such rigidity was found by PULLING[8] in the shallow root systems of *Picea mariana*, *Larix laricina*, and *Betula alba papyrifera*, as well as in the more deeply rooted *Pinus Strobus* and *P. Banksiana;* while both the shallow rooted *Picea canadensis* and the deep rooted *Populus balsamifera* exhibited considerable plasticity.

CANNON[9] believes that the roots of deeper penetration are less responsive to changes in aeration and temperature than those of more superficial habit, basing his conclusion upon the study of *Pistacia atlantica* and *Prosopis velutina* of the former class, and *Opuntia versicolor* and *O. discata* of the latter class. The individuality of such responses is further shown by the studies of CANNON and FREE,[10] proving that while certain plants like *Opuntia* stop root growth with a soil atmosphere of 50 to 75 per cent carbon dioxide, others, like *Prosopis*, continue growth as long as 2 per cent of oxygen is

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[6] MARKLE, M. S., Root systems of certain desert plants. BOT. GAZ. **64**:177–205. *figs.* 33. 1917.

[7] WATERMAN, W. G., Development of root systems under dune conditions. BOT. GAZ. **68**:22–53. *figs.* 17. 1919.

[8] PULLING, H. E., Root habit and plant distribution in the far north. Plant World **21**:223–233. *fig.* 1. 1918.

[9] CANNON, W. A., Modifications of root habits by experimental means. Carnegie Inst. Wash. Yearbook **17**:83–85. 1919.

[10] CANNON, W. A., and FREE, E. E., The ecological significance of soil aeration. Science N.S. **45**:178–180. 1917.

present. They also showed that while the roots of *Coleus blumei* and *Heliotropium peruvianum* show injury in 3 days by an addition of 25 per cent nitrogen to the soil atmosphere, *Nerium oleander* is unharmed by 50 per cent of nitrogen, and the roots of *Salix* (*nigra?*) grow freely in pure nitrogen. Similar results were obtained by the use of helium instead of nitrogen as a diluting gas.

More recently BERGMAN[11] has found similar differences of response in the roots of land and swamp plants, the dead roots in the former often being replaced by others near the surface of the water, showing lack of aeration to be one of the most important factors involved. Several experiments serve to give emphasis to this fact. He found that land plants with submerged roots soon show pronounced wilting, the wilting being less marked when the submergence is in aerated water, and a reduction in transpiration preceding wilting. This is taken to indicate that absorption is reduced below the amount demanded by transpiration. When aeration is provided, the use of swamp water for submergence or watering gives no other harmful results than those obtained by the use of tap water or nutrient solutions. The oxygen content of swamp water in nature was found to be large in the open lakes examined, but to show decided decrease through the *Carex* stages to the *Chamaedaphne-Andromeda* and *Larix-Picea* stages. This leads to the conclusion that the mingling of hydrophytes, mesophtyes, and xerophytes in swamps is due to local differences in habitat, such as water level and aeration, affecting the rate of absorption and its ratio to transpiration; hence ecesis in swamps can occur only when the oxygen requirements of the species are satisfied.

These citations show that considerable descriptive matter has added materially to our knowledge of root systems, and that the few physiological investigations of these organs have pointed to wide diversity in the responses of individual species to changes in their environment.—GEO. D. FULLER.

Alpine vegetation of the central Andes.—HAUMAN[12] has recently described a scanty alpine vegetation found on the Andes between 31 and 37° south latitude, at elevations ranging from 2000 to 42,000 m. This region possesses many peaks above 6000 m. high, the highest and best known being Aconcagua, with an altitude of 7020 m. These mountains are snowcapped and possess a good development of glaciers, from which flow tortuous and variable streams, furnishing almost the entire water supply for the sparse vegetation, since the growing season in these mountains is almost entirely without rain. The temperature records are imperfect, but an important factor is the light frosts,

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[11] BERGMAN, H. F., The relation of aeration to the growth and activity of roots and its influence on the ecesis of plants in swamps. Ann. Botany **34**:13–33. *fig. 3.* 1920.

[12] HAUMAN, LUCIEN, La végétation des hautes cordillères de Mendoza (République Argentine). Anales Soc. Cien. Argentina **86**:121–188. *pls. 5–22. figs. 7.* 1918.

which are common throughout the growing season. One station at 2700 m. gives an annual mean temperature 6.5, with a mean maximum of 13.4 and a mean minimum of 0.1° C. Humidity at all times is low, while wind velocity is decidedly high and constant. Precipitation as recorded at 2000 m. seems to be irregular and variable, the annual amounts ranging from 20 to 68 cm., occurring principally in the colder months in the form of snow. This deficiency of rainfall, combined with other factors, makes the vegetation not only very scanty, but limited to valleys and slopes which possess streams or seepage water from the glaciers and snowfields. In the absence of mountain lakes aquatic vegetation is scanty, and anything resembling mountain meadows is limited to the stream edges and small alluvial fans. Such grassy associations appear to resemble closely similar alpine areas elsewhere. Related to the alpine meadows are the "high Andean oases," formed at 3200 to 3600 m., where at the foot of talus or morainal slopes some alluvial soil has accumulated. These oases vary in size, but rarely reach 100 m. in diameter. They are often dominated by the juncaceous *Andesia bisexualis* 15 to 30 cm. high, forming a thick carpet.

Trees are absent throughout, and even in the valleys the shrubs do not exceed 2 m. in height. *Adesmia pinifolia* (a legume) is the most plentiful shrub; while among the others are *Ephedra americana andina*, *Berberis empetrifolia*, and *Senecio uspallatensis*. *Opuntia andicola*, the only cactus of the region, together with *Azorella Gilliesii* and *Laretia acaulis*, two umbellifers, form a curious trio of herbaceous cushion plants confined to the valleys.

Upon the more exposed parts of the mountains there is a notable abundance of prostrate, tufted, rosette, and cushion plants, often with a striking development of large woody roots. These growth forms are accounted for as being a response to exposure to high winds and dependence upon a subterranean water supply. Upon the slopes *Adesmia trijuga*, with shrubby cushions 30 cm. high, together with *Poa chilensis* and *Stipa speciosa* in tufts, dominate the area, forming scattered dots over the rocky landscape. Most abundant upon the summits between 3000 and 4000 m. are the subterranean woody cushions of *Adesmia subterranea*, whose leaves form a carpet upon the surface. Accompanying this species with similar growth forms are the more uncommon *Verbena uniflora* and *Oxalis bryoides*.

The entire vascular flora consists of 417 species, including one pteridophyte, *Cystopteris fragilis*, and one gymnosperm, *Ephedra*. Among the richest families are Compositae with 85 species, Leguminosae with 36, Gramineae with 34, Cruciferae with 28, Portulacaceae with 15, Umbelliferae with 15, Rosaceae with 12, Cyperaceae with 12, Oxalidaceae with 10, and Violaceae and Caryophyllaceae with 9 species each. Large genera are *Senecio* with 26 species, *Adesmia* with 16, *Calandrinia* with 15, *Astragalus* with 12, *Oxalis* with 10, and *Viola* with 9 species. The scarcity of the Saxifragaceae, with two rare species, and the entire absence of the Ericaceae and Primulaceae are worthy of note. Lichens, abundant at the lower altitudes, become very rare

above 2800 m.; mosses are common about springs up to 3600 m., but liverworts are entirely lacking. More than one-half the species (210) are classed as belonging to the central Andes, 60 being endemic. There are no endemic genera, but notable among this group are such aggregates as 6 species of *Adesmia*, 2 of *Boopis*, 12 of *Senecio*, and 2 new varieties of *Koeleria*. The other elements are the northern tropical with 16 species, the subtropical with 21 species, the basal Argentinian with 56 species, the southern Andean with 10 species, the Patagonian with 73 species, and the cosmopolitan and introduced species numbering respectively 28 and 17. This introduced element must be regarded as small when it is recalled that the Mendoza River valley has been the trans-Andean route for centuries.

Photographs and careful drawings of many of the interesting forms add much to the value of the report.—GEO. D. FULLER.

Crop centers.—A great service in unifying ecology and agriculture has recently been rendered by WALLER,[13] who has illustrated by well chosen examples the close relation that exists between crop and vegetation centers. TRANSEAU has shown how closely vegetation centers are indicated by a map showing the ratio of rainfall to evaporation, and WALLER now emphasizes the fact that corn, wheat, and similar crops show strikingly similar relations. It is often said that crops are moving west or north, which merely means for the most part that we are finding their range. For example, wheat was first cultivated away from its proper center, so that in the last 70 years the center of wheat cultivation has moved 700 miles west and 100 miles north. A fundamental difference between crops and native plants is that when the latter extend far beyond their range, it is chiefly in the poorest soil, since competition with plants proper to the district exclude them elsewhere. Crops grown at the edge of their range, however, must be grown in the best conditions available, and of course are exempt from competition. Special attention is paid to corn, wheat, and cotton, and the maps showing their distribution are very significant. Of course there are many complexities in working out the thesis. Economic considerations, such as problems of market and transportation, figure very largely. Considering its origin, the center of corn might be sought south; competition with cotton is thought to be the major factor here. The dominance of eastern Illinois in corn production, and of North Dakota in the production of spring wheat, are related to edaphic factors; in each case there is rich prairie soil.—H. C. COWLES.

Increasing catalase activity in yeast cells.—EULER and BLIX[14] have determined the effect of various conditions and reagents upon the catalase activity

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[13] WALLER, A. E., Crop centers of the United States. Jour. Amer. Soc. Agron. **10**:49–83. *figs. 8*. 1918.

[14] EULER, H. V., and BLIX, R., Verstärkung der Katalasewirkung in Hefezellen. Hoppe-Seyler Zeit. Physiol. Chem. **105**:83–114. 1919.

of yeast cells. When possible they used the potassium permanganate titration method for determining catalase activity. In cases where additions of thymol, glucose, etc., rendered the permanganate method inaccurate, the volumetric method was used. They used mainly their cultures of distillery top yeast S.B. II. Some experiments were run with brewery bottom yeast. They agree with PHRAGMEN's findings that yeast splits dilute solutions of hydrogen peroxide without secreting a soluble enzyme into the bathing fluid. The reaction is one of the first order. The reaction constant increases in proportion to the amount of yeast. Small amounts of protoplasmic poisons (toluol or chloroform) raise the catalase activity of these cells 6-fold. When cells were dried in the air or otherwise without injuring them, the catalase activity rose 10–15-fold. When emulsions of the yeast were heated 0.5–2 hours at 55–63° C., the catalase activity rose 20–30-fold. The activation by heating is greatly influenced by reagents in the emulsion at the time of heating. Similar activation of catalase has been demonstrated in a number of other micro-organisms. The catalase activity of yeast can be raised by previous treatment with sugar solutions. This increased catalase activity is not due to increased permeability of the cells to catalase, but is an activation within the living cells. The reaction constant is not a measure for the catalase content of the cells.—WM. CROCKER.

Parasitism.—HAWKINS and HARVEY[15] have made an interesting study of the nature of the resistance of White McCormick tubers to the tuber rot caused by *Pythium debaryanum* Hesse. The White McCormick is very resistant to the disease, while Bliss, Triumph, and Green Mountain are very susceptible. From their experiments they think it probable that the fungus enters the cells of the potato by mechanical puncture of the cell walls and not by enzyme action. The McCormick is less susceptible to the disease than the other varieties, because its cell walls are more resistant to this mechanical puncture. Determinations of the pressure required to puncture the cell walls give much higher results for the McCormick than for the susceptible varieties. The rate of growth of the fungus is much slower in the McCormick. Correlated with the greater resistance of the McCormick is a higher crude fiber content. If its osmotic pressure is to be considered the force available to the fungus for this mechanical puncture of the cell walls, then the cases of resistance of the potatoes used in the experiments would be explained, with three exceptions.—S. V. EATON.

Correlations.—CHILD and BELLAMY[16] have done a very interesting piece of work on correlations in plants. They can break up correlation effects by

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[15] HAWKINS, L. A., and HARVEY, R. B., Physiological study of the parasitism of *Pythium debaryanum* Hesse on the potato tuber. Jour. Agric. Res. **18**:275–297. *pls. 35–37. figs. 2.* 1919.

[16] CHILD, C. M., and BELLAMY, A. W., Physiological isolation by low temperature in *Bryophyllum* and other plants. Science **50**:362–365. 1919.

cooling 2–3 cm. zones of petioles and stems to a temperature of 2.5–3° C. In *Bryophyllum*, when such zones of the petiole are cooled, the broken correlation is manifested by development, not only in the notches of the leaf treated, but by development in the notches of the opposite leaf, as well as leaves both up and down the stem. The effect extends farther in the basal direction than in the apical. This indicates marked complexity in the correlation inhibitive effects. In *Phaseolus* the axial buds below the cooled zone grew. In *Saxifraga sarmentosa* the runner tip could be thus isolated. All of these results favor McCallum's view that correlative effects are brought about by conduction of stimuli, mainly inhibitory stimuli, and not by movements of materials.—Wm. Crocker.

Fermentation.—Euler and Svanberg[17] made a study of alcoholic fermentation in an alkaline medium in which P=8. Top yeast and *Torula* gave about equal weights of carbon dioxide and alcohol, each equal to 30–33d of the weight of the sugar fermented. Glucose, fructose, and invert sugar were fermented with about equal speed, mannose about 30 per cent as fast, and galactose very slowly. Invertase is active in this medium and maltase inactive. The following are the maximum alkalinities in which cell division occurs in the various yeasts: Frohberg Unterhefe B., $P_H=7.7$–8; Brennerei Oberhefe S.B. II, $P_H=7.3$–8.4; *Sacch. ellipsoideus*, $P_H=7.9$; *Pseudosacch. apiculatus*, $P_H=7.6$. Increase in weight occurred in S.B. up to $P_H=8.5$. For Frohberg Unterhefe H the full curve of acid sensitivity was worked out and the optimum was found to be at $P_H=5$.—Wm. Crocker.

Exudation of water by leaves.—Miss Flood[18] has recently investigated the exudation of extremely pure water by the leaf tips of *Colocasia antiquorum*. Examination of sections of leaf tips showed no membrane, or other structure which might act as a filter, between the vascular system of the leaf blade and the pores leading to the tip. Solutions of India ink, gelatine, and starch were forced through the vascular system and exuded at the tips. Exudation from leaves attached to the plant continued at the normal rate when leaf tips were anaesthetized. Miss Flood is of the opinion that cells lower down in the plant are responsible for the secretion and filtration of water, but finds no evidence for the existence of such cells except in the root.—J. M. Arthur.

Colorado grasslands.—Reviewing the investigations of the grasslands of Colorado by himself and others, Ramaley[19] enumerates all the associations

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[17] Euler, H., and Svanberg, O., Enzymatische Studien über Zuckerspaltungen. Hoppe-Seyler Zeit. Physiol. Chem. **105**:187–239. 1919.

[18] Flood, Margaret G., Exudation of water by *Colocasia antiquorum*. Proc. Roy. Dublin Soc. (N.S.) **15**: *pls. 2*. 1919.

[19] Ramaley, Francis, Xerophytic grasslands at different altitudes in Colorado. Bull. Torr. Bot. Club **46**:37–52. *figs. 2*. 1919.

that have been described. He also gives a brief synopsis of the factors most prominent in the control of such vegetation, and some of the more important floristic differences which characterize the grasslands at different altitudes. A notable reduction of species is manifest with increase of altitude, the estimate running from 160 species for the mesas, 139 for the foothills, and 107 for the montane, to 50 for the subalpine. A systematic list of species is given with indications of their occurrence at different altitudes. The whole, including the bibliography, forms a most useful contribution, summarizing the present state of our knowledge of these plant communities.—GEO. D. FULLER.

Biology of Fomes.—WHITE[20] has made a comprehensive study of the widely distributed *Fomes applanatus*, and finds that it attacks practically all deciduous trees and several conifers, causing the destruction of large quantities of wood annually. It produces basidiospores only, which are not of the ordinary type, being "yellow, papillate, thick-walled chlamydospores within a thin hyaline wall." Spore discharge is enormous and continues for a longer period than recorded for any other fungus, being continuous day and night for about 6 months. There was no difficulty in making artificial cultures, and the appearance of the rotted wood makes it possible to distinguish the attack of this fungus from that of any other form. The histological and chemical details of the attack are fully described.—J. M. C.

Ecology of fungi.—Studying the influence of altitude upon parasitic fungi from collections made by FRAGOSCO in Cataluña, Spain, and by himself in Barreges, DUFRENOY[21] found that the Pyrenees are not a barrier to the dissemination of fungi, although there are certain differences between the fungus flora of the closely adjacent parts of France and Spain. He concludes that there are species peculiar to the plains and to the mountains, as well as those common to both habitats. The determining factor in altitudinal distribution seems to be neither humidity nor temperature, but radiation. The mountain species are either more highly colored or are found on more highly colored hosts. He was unable to determine any effect of altitude upon the resistance of the host.—GEO. D. FULLER.

Pennsylvania trees.—The fact that within 5 years ILLICK'S[22] tree manual has reached its third edition is a striking testimony to its excellence. The first part of the volume is devoted to a general discussion of forests, their structure, development, care, and value receiving careful consideration, and

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[20] WHITE, J. H., On the biology of *Fomes applanatus* (Pers.) Wallr. Trans. Roy. Can. Inst. Toronto 1919: 133–174. *pls. 2–7.*

[21] DUFRENOY, J., Les conditions écologiques du développement des champignons parasites. Etude de géographic botanique. Bull. Soc. Mycol. France **34**:8–26. 1918.

[22] ILLICK, J. S., Pennsylvania trees. 3d ed. pp. 235. *pls. 1–129. figs. 120.* Harrisburg: Dept. Forestry Penn. Bull. **11**: 1919.

is illustrated by many very appropriate photographs. The form and structure of trees are also carefully considered. The second part is devoted to a manual of the trees of the state, and is well equipped with keys, glossary, and illustrative drawings. A noticeable feature of the illustrations of the individual species is the drawing of the buds on a large scale. It is safe to say that it will take a first rank among the numerous tree manuals now available.—GEO. D. FULLER.

Montane plants of the Rocky Mountains.—RYDBERG,[23] in continuing his studies of the flora of the Rocky Mountains, has added to the articles already noted in this journal[24] an investigation of the distribution of the montane species. He finds about 1900 species in this zone, of which one-half are to be regarded as typical inhabitants of this area. Less than 15 per cent are transcontinental, while 53 per cent are endemic. A close analysis is made of the constituents of the flora peculiar to the northern and southern portions of the region as contrasted with that common to both.—GEO. D. FULLER.

Sedge associations in Colorado.—In studying the sedges of northern Colorado, RAMALEY[25] shows that the genus *Carex* not only is of decided importance, but that species of this genus dominate many plant associations, particularly in the montane, subalpine, and alpine regions. These associations are either hydrophytic or xerophytic in character, and represent early stages in succession, for as mesophytism is approached the sedges are replaced by grasses and dicotyledons. The principal associations involved are briefly described and their sedge components noted. Of the 44 species of *Carex* listed, 20 are classed as hydrophytic, 15 as xerophytic, and 9 only as mesophytic.—GEO. D. FULLER.

New African plants.—ENGLER,[26] in continuation of his studies of the African flora, has described 45 new species of Sterculiaceae, 40 of which belong to *Hermannia*, 29 new species of Guttiferae, and 3 new species of Violaceae (belonging to *Hybanthus*).—J. M. C.

A new genus of Umbelliferae.—THELLUNG[27] has described a new genus (*Scandicium*) of Umbelliferae from the Mediterranean steppe region and Western Asia, based on *Scandix stellata* Solander. In addition to the species, numerous varieties are described.—J. M. C.

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[23] RYDBERG, P. A., Phytogeographical notes on the Rocky Mountain region. VIII. Distribution of the montane plants. Bull. Torr. Bot. Club **46**:295–327. 1919.

[24] BOT. GAZ. **62**:83–84. 1916; **63**:423–424. 1917; **65**:195. 1918.

[25] RAMALEY, FRANCIS, The rôle of sedges in some Colorado plant communities. Amer. Jour. Bot. **6**:120–130. *fig. 2*. 1919.

[26] ENGLER, A., Beiträge zur Flora von Afrika. XLVII. Bot. Jahrb. **55**:350–400. 1919.

[27] THELLUNG, A., *Scandicium*, ein neues Umbelliferen-Genus. Sonderabdruck aus Fedde, Repertorium **16**:15–22. 1919.

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THE
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TEMPERATURE AND RATE OF MOISTURE INTAKE IN SEEDS[1]

CHARLES A. SHULL

(WITH FOUR FIGURES)

Introduction

Some years ago BROWN and WORLEY (1) published an account of some experiments dealing with the influence of temperature on the rate of moisture intake by seeds of barley. They found that the value of Q_{10} for the intake of water is high, approximating that of the van't Hoff law. They interpreted this as indicating that the rate of water absorption through a semipermeable membrane is conditioned by some chemical change which occurs as the temperature rises. In discussing the probable nature of this change they intimate that the water molecule is probably simplified as a result of the temperature rise. In doing so they in a measure accept ARMSTRONG'S hydrone theory of the structure of water. Cold water, according to this conception, is composed of complex molecules having at least several H_2O groups combined into a single molecule. These more complex molecules are supposed to break down into simpler groups as the temperature rises; the water becomes less viscous, and is able to penetrate the semipermeable coats of barley seeds more rapidly. The velocity of water intake

[1] Contributions from the Botanical Laboratories of the University of Kentucky, no. 1.

was calculated from the tangents of the curves of intake, using a string and protractor for measuring the tangents. This is a very crude and inaccurate method, especially in unskilled hands, but one easily used. They assert that the velocity of water absorption is almost exactly an exponential function of the temperature.

A short time previous to the appearance of this work the writer (3) had found that the seeds of *Xanthium* have semipermeable coats, and experiments on the influence of temperature on the rate of moisture intake by these seeds were in progress at about the time that BROWN and WORLEY'S paper appeared. The results of the work, however, did not receive careful mathematical consideration until about two years later, when it was found that the conclusions reached by BROWN and WORLEY from their work on barley seeds could not be drawn from the data which had been obtained from *Xanthium* seeds. A preliminary report of the work was made before the Botanical Society of America at the Columbus meeting in 1915. The data which had been obtained indicated that the value of Q_{10} was approximately 1.5, somewhat higher than the temperature coefficient of diffusion, but notably lower than that of chemical processes. This situation is very similar to that later reported by DENNY (2) for the effect of temperature on the rate of permeability of certain plant membranes to water.

Shortly following the Columbus meeting a few tests were run on seeds of *Xanthium* having a somewhat different environmental history. Mainly, the seeds were older than those previously used. The intake curves did not check very well with the former data, and it was thought desirable to repeat the experiments with seeds of the same species of *Xanthium* but of different genetic origin and environmental history. In this way it was felt that data might be obtained regarding the variability in the rate of water absorption in these seeds. The data which have been accumulated have been subjected to a critical analysis, principally to insure accuracy in the measurements of tangents. At the same time the possibility of a rate law has been kept in mind; but from a study of absorption in a number of cases I have decided that it would be unsafe or at least premature to propose a rate law on the basis of data now obtained. At the same time, the formulae presented may have

rather wide application, and deserve to be considered by those interested in the problems of absorption. While on the theoretical side certain features of the work have been disappointing, it will be worth while to give a somewhat detailed account of the experiments, as a contribution to our knowledge of the facts concerning the intake of water by dry organized matter.

I wish to acknowledge my indebtedness to Professor S. P. SHULL for valuable assistance with the mathematical part of the work. He has given generously of his time during the last five years to a painstaking analysis of the data, which has made possible a degree of accuracy otherwise unobtainable, and without which the general significance of the data could not have been fully appreciated. He has also tested many hypotheses as to the influence of factors upon intake rates. The principal part of the experimental work was done in the Laboratory of Plant Physiology at the University of Kansas, and part of it at the University of Chicago during the summer of 1914. The privileges of the Hull Botanical Laboratory for this work were much appreciated.

Materials and methods

The experiments were carried on with the lower seeds of *Xanthium pennsylvanicum* Wallr., and the naked cotyledons of several varieties of peas, the Canada green field pea, the Tom Thumb garden pea, and the Small Scotch Yellow pea of commerce. The cockleburs were chosen for their semipermeable coats, and the peas because the elimination of coat effects is easy. At first seeds of *Xanthium* were collected in the field; but these were soon replaced by pure line seeds grown on the breeding grounds of the University of Kansas in 1913. It was felt that such seeds might be more valuable than those of mixed genetic origin, more uniform in behavior, and the absorption data therefore more susceptible to mathematical consideration. After it had become evident that age, environmental history, genetic origin, and other factors might influence the intake phenomena, seeds were obtained from plants growing near the writer's home in Lawrence, Kansas. Slight differences in the shape and appearance of the seeds of different plants indicated possible lack of genetic purity, although the

plants by all their external characteristics were unmistakably true *X. pennsylvanicum*. These were used in the later work to give an idea of the variability to be encountered in the moisture intake by a given kind of substance.

The absorption took place in test-tubes of distilled water which were kept at the desired temperature by standing them in a water bath. Care was taken, particularly in the later work, to have the seeds at the same temperature as the water when they were first brought together. Three temperature curves are discussed in the present paper, 5, 20, and 35° C. Tests were run at 5° intervals from 5° to 50° C., but these three stand near to the temperatures used by BROWN and WORLEY, and afford a satisfactory basis for comparison. The others have been omitted. In all cases the fluctuation rarely exceeded 0.25° on either side of the chosen temperature during the significant period of intake.

At close intervals the seeds were removed from the water, dried uniformly and quickly on filter paper, and weighed with analytical accuracy. The time periods of immersion were made as sharp and accurate as possible, and the time during which the seeds were out of the water was reduced to the lowest possible limit. The drying required 10–20 seconds usually, and the weighing was done as rapidly as accuracy permitted. During this period the seeds had some opportunity to change from the temperature of absorption in the 5° and 35° tests, but histeresis of the seed colloids would tend to prevent serious alterations in colloidal aggregation during the brief interval involved. The errors due to such changes would be slight. The intervals between weighings were made short throughout the work. The first weighing was always made at the end of 1 minute to catch the very rapid initial intake. Succeeding intervals were usually 10 or 15 minutes, or longer when continuous attention could not be given to the work. The time intervals used will always be indicated in the tables with the absorption data. In all cases the time needed for drying and weighing was subtracted. This weighing at intervals was continuous in the case of *Xanthium* seeds until the intake was well above 35 per cent out of a possible 50–55 per cent. By the time 40 per cent of water had been taken in, the velocity of intake always

showed marked and increasing depression, due to approaching saturation. The split peas take up a considerably larger percentage of water than *Xanthium* seeds, and the intervals were continued until intake significant for the problem in hand had ceased.

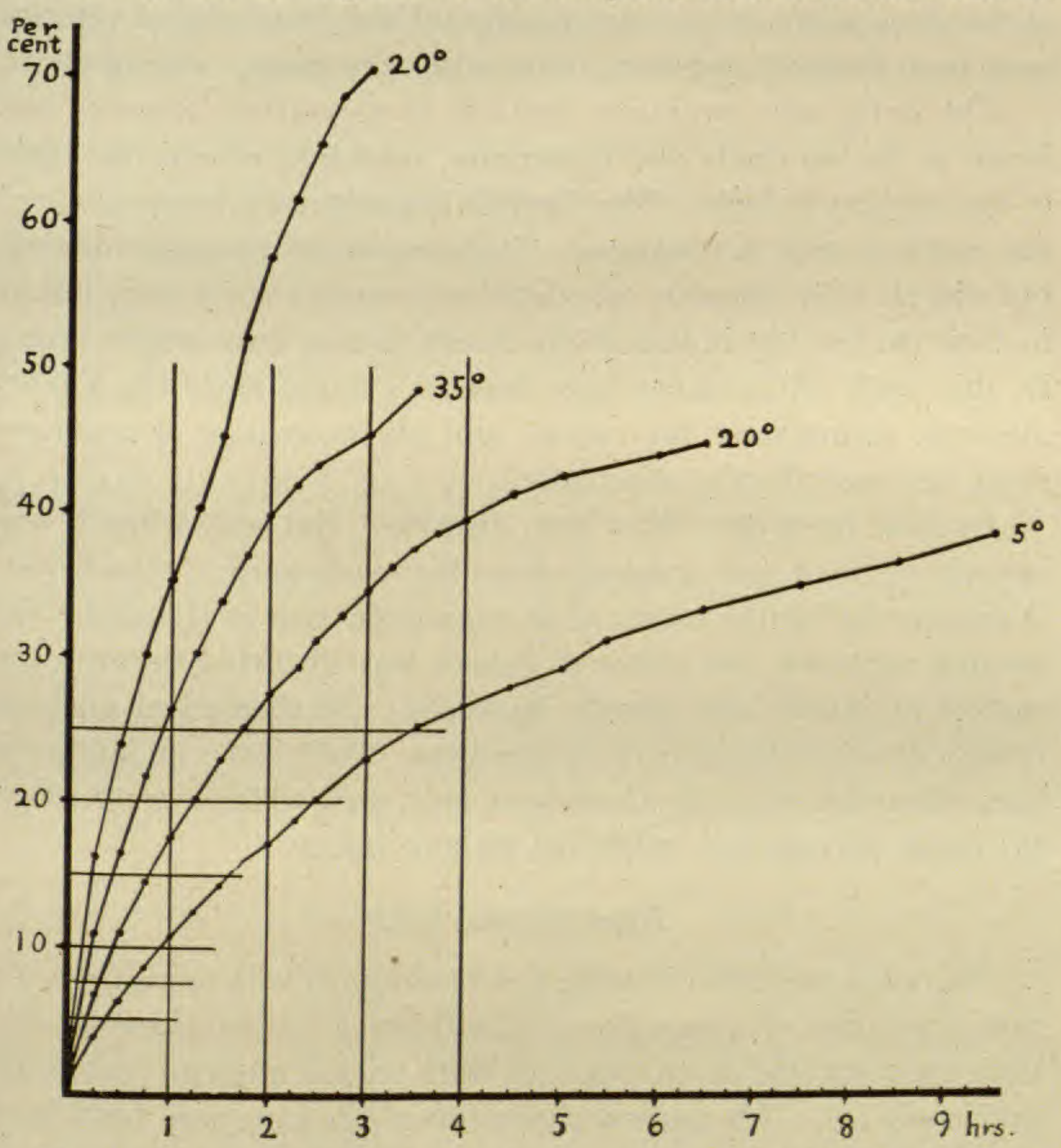

FIG. 1.—Curves of moisture intake: lowest curves, 5, 20, and 35°, by *Xanthium* seeds; upper curve, split peas, 20°; horizontal lines show points of equal intake where tangents were measured.

The value of close time intervals, despite certain obvious disadvantages, will be indicated later in discussing the work of BROWN and WORLEY.

The velocity of intake at any given moment has been calculated from the tangents to the curves. By reference to fig. 1 it will be

seen that horizontal lines cut the three temperature curves for *Xanthium* seeds at 5, 7.5, 10, 15, 20, and 25 per cent of intake. The tangents were determined at the points where these horizontal lines of equal intake cut the curves. From the velocity of intake at the three points cut by each horizontal line, the ratios of velocity have been derived, and from these ratios the mean value of Q_{10}.

The string and protractor method of measuring tangents was found to be too crude and inaccurate, especially where the angle of the tangent is high. The English investigators, however, used the method with fair success. Their measured tangents deviate but slightly from tangents calculated accurately for the same points in their curves, but in less skilful hands serious error might occur. In this work all tangents have been calculated from the known algebraic formulae of the curves, and all inaccuracy of measurement has been thereby eliminated.

In some cases data have been discarded, but only when it was entirely justified, and necessary from the mathematical standpoint. Whenever during the course of an experiment any of the seed coats became ruptured, the curve of intake was distorted because the surface of intake was greatly increased. Mathematical analysis of such data is impossible or meaningless. Such series of data have been discarded, and only those have been used which went through the many dryings and weighings without injury.

Experimental data

The data presented in table I were obtained with seeds from the first generation of a pure line of *Xanthium pennsylvanicum* Wallr., from the same line as was used for work on soil moisture published previously (4). The general characters of the type used have been described as type II in a discussion (5) of physiological isolation in the genus. The series of data chosen for mathematical consideration were drawn from a large mass of data some time before the analysis was made, solely on the basis of maintenance of satisfactory conditions during the period of observation. Ten lower seeds of *X. pennsylvanicum* were used in each case.

The series at any given temperature were fairly uniform with these seeds at the time the work was done. The variability to

be encountered is illustrated very well by the duplicate tests presented for 5° C.

The earlier work on split peas was not very satisfactory. They are more difficult to dry uniformly, and small pieces are more easily lost from the edges of the cotyledons during the drying, especially at higher temperatures. In table II data are given for two tempera-

TABLE I

WATER INTAKE OF *Xanthium* SEEDS IN PERCENTAGE OF AIR-DRY WEIGHT

TIME	5°		20°	35°
	I	II	I	I
1 minute	1.124	1.36	1.73	2.45
15 minutes	3.814	4.23	6.806	10.89
30 minutes	6.226	6.18	11.00	16.41
45 minutes	8.544	8.32	14.55	21.81
60 minutes	10.747	9.92	17.38	26.38
75 minutes	12.521	11.90	20.20	30.21
90 minutes	14.202	13.05	22.81	33.89
105 minutes	15.710	14.65	25.12	37.11
120 minutes	17.101	15.81	27.44	39.80
135 minutes	18.724	17.65	29.32	41.87
150 minutes	20.182	19.81	31.06	43.25
165 minutes	.....	20.90	32.80	.....
180 minutes	23.002	22.27	34.54	45.24
195 minutes	.....	23.40	36.13	.....
210 minutes	25.159	24.54	37.43	48.46
225 minutes	.....	26.54	38.52	.....
240 minutes	26.701	.....	39.39	.....
255 minutes	.....	27.96	.....	.....
270 minutes	27.965	.....	40.98	.....
290 minutes	.....	29.56	.....	.....
300 minutes	29.182	.....	42.57	.....
330 minutes	31.304	31.00	.....	.....
360 minutes	.....	.....	43.95	.....
390 minutes	33.159	.....	44.75	.....
450 minutes	35.072	.....	.....	.....
510 minutes	36.486	.....	.....	.....
570 minutes	38.400	.....	.....	.....
18.5 hours	45.020	.....	.....	.....
26 hours	.....	.....	47.28	.....

tures only, 20 cotyledons being used for each measurement. Curves of intake have been plotted for the cocklebur seeds at all three temperatures, and for the split peas at 20° C. in fig. 1. The split peas are included here merely to show how various substances differ in rate of intake at the same temperature. The rate of

intake, of course, varies with physical structure, chemical composition, state of aggregation of colloids, etc.

TABLE II

WATER INTAKE OF COMMERCIAL SPLIT PEAS (VARIETY UNKNOWN) IN PERCENTAGE OF AIR-DRY WEIGHT

Time	20°	35°
1 minute	4.25	5.30
15 minutes	16.20	20.83
30 minutes	23.84	33.04
45 minutes	30.14	41.94
60 minutes	35.32	50.30
75 minutes	40.30	57.60
90 minutes	45.22	63.77
105 minutes	52.06	67.81
120 minutes	57.50	71.00
135 minutes	61.42	.....
150 minutes	65.34	.....
165 minutes	68.66	74.32
180 minutes	70.32	.....
195 minutes	72.18	.....
210 minutes	72.97	.....
285 minutes	.....	76.11
330 minutes	74.96	.....
22 hours	.....	77.50
22.5 hours	74.96	.....

TABLE III

WATER INTAKE BY *Xanthium* SEEDS IN PERCENTAGE OF AIR-DRY WEIGHT, 5° C.

Time (minutes)	I	II	III	IV	V	Percentage of averages
1	1.30	1.90	1.33	2.19	1.57	1.64
5	3.29	3.25	3.70	3.87	3.33	3.44
10	4.74	4.47	5.25	5.92	4.90	4.97
15	6.20	5.55	6.88	6.87	6.15	6.22
30	8.49	8.13	9.02	9.72	8.92	8.72
45	10.79	10.43	11.09	11.76	10.87	10.82
60	12.85	11.79	13.02	13.00	12.81	12.50
90	15.68	14.50	15.53	16.73	15.89	15.43
120	18.36	16.87	18.05	18.85	18.09	17.77
150	21.04	18.97	20.56	20.67	20.29	19.99
180	22.88	20.73	22.71	22.42	22.30	21.86
240	26.55	24.39	25.89	26.08	26.26	25.42
300	30.22	27.51	28.99	29.07	29.71	28.67
360	33.59	30.28	32.03	31.92	32.91	31.67
420	37.18	32.25	34.91	35.14	35.30	34.55
540	40.93	36.86	39.20	39.96	40.70*	38.56

* During the last two hour period in no. V the mean temperature was about 6.2° C.

After the earlier work had been analyzed, some tests were made on old seeds remaining on hand, in an effort to check up the initial

absorption rates. As the seeds seemed to show a somewhat different behavior, tending to decreased intake rates at the start, it was

TABLE IV

WATER INTAKE BY *Xanthium* SEEDS IN PERCENTAGE OF AIR-DRY WEIGHT, 20°C.

Time (minutes)	I	II	III	IV	V	VI	Percentage of averages
1	1.77	1.95	3.32	2.35	2.72	2.08	2.38
5	4.60	5.28	6.15	5.56	5.44	4.94	5.34
10	6.76	8.21	8.59	8.26	8.21	7.41	7.94
15	8.44	11.44	10.45	11.07	10.28	10.27	10.32
30	11.79	13.88	14.69	14.98	16.51	16.51	14.64
45	15.10	17.15	17.97	18.83	22.15	20.68	18.54
60	17.64	20.33	20.51	21.53	23.84	23.67	21.13
75	19.94	22.29	22.95	24.29	26.90	26.27	23.64
90	21.81	24.63	25.78	26.79	29.18	28.87	26.04
105	23.78	26.88	27.56	29.19	31.21	31.21	28.17
120	25.89	28.84	29.88	31.10	32.59	33.42	30.14
135	28.00	31.33	31.69	32.95	34.47	35.63	32.16
150	29.53	32.50	33.20	34.30	36.10	37.84	33.72
165	31.35	34.31	34.56	35.75	37.09	39.27	35.20
180	32.89	35.48	35.84	37.20	38.77	40.96	36.66
195	34.32	36.46	36.91	38.11	39.61	42.46	37.77
210	.....	37.54	37.79	39.01	40.50	43.69	38.79
225	.....	.....	38.86	40.01	41.44	44.73	.....
240	37.68	.....	39.45	40.86	42.33	45.77	40.64
300	40.27	41.84	41.55	44.56	.....	.....	.....

TABLE V

WATER INTAKE BY *Xanthium* SEEDS IN PERCENTAGE OF AIR-DRY WEIGHT, 35° C.

Time (minutes)	I	II	III	IV	V	VI	VII	VIII	Percentage of averages
1	2.21	2.46	3.73	2.84	2.46	2.75	3.76	2.44	2.83
5	6.35	7.37	9.28	7.61	5.88	6.19	9.92	7.04	7.46
10	10.22	11.37	15.40	11.34	9.78	10.25	14.98	11.10	11.82
15	14.23	14.59	20.24	14.25	12.31	12.79	18.47	13.80	15.11
20	16.85	17.36	23.13	17.15	14.09	15.34	21.41	16.51	17.84
30	21.28	22.58	27.77	21.85	19.49	19.95	25.44	21.25	22.45
40	25.14	26.42	31.94	25.45	23.67	23.52	28.86	25.44	26.32
50	28.80	30.18	34.90	29.25	27.09	26.82	31.81	26.69	29.70
60	32.39	33.79	37.66	32.85	30.16	30.12	34.68	31.94	32.95
70	35.15	36.64	40.01	35.20	33.11	33.29	37.21	35.05	35.71
80	37.29	39.48	42.23	37.76	35.50	36.31	39.33	37.21	38.13
90	39.23	41.78	44.05	39.70	37.62	38.79	41.18	39.24	40.19
100	39.50	43.78	45.53	41.22	39.67	40.92	42.75	41.00	41.78

felt that studies should be made of absorption in ordinary field material, with the purpose of disclosing the variability likely to occur at a given age of seeds. These experiments were conducted

through a period of several weeks on seeds ripened for about three months. To reduce the time element in drying and weighing, only two seeds were used in each test. At the same time care was taken to have the temperature of seeds and water equal at the beginning of the measurements in each test. Table III shows the results of

TABLE VI

WATER INTAKE IN SPLIT PEAS IN PERCENTAGE OF AIR-DRY WEIGHT

Time (minutes)	Tom Thumb Yellow			Green Canada field pea			Small Scotch Yellow		
	5°	20°	35°	5°	20°	35°	5°	20°	35°
1	3.00	3.76	4.38	3.17	4.09	5.54	1.77	5.38	5.77
5	7.28	8.60	10.19	7.98	9.20	14.01	7.26	13.26	16.34
10	10.48	12.63	14.90	11.73	13.49	20.30	11.50	19.98	26.54
15	13.05	15.50	18.69	14.61	16.68	25.62	15.58	25.09	33.65
20	.....	.....	22.10	.....	.....	30.94	.....	.....	41.54
30	16.79	21.15	27.78	19.33	22.98	41.59	22.48	33.69	54.23
40	.....	.....	32.24	.....	.....	52.33	.....	.....	63.75
45	20.02	26.16	.....	23.08	28.78	.....	26.64	.....	.....
48	.....	.....	.....	.....	.....	.....	.....	43.01	.....
50	.....	.....	36.78	.....	.....	68.24	.....	.....	69.04
60	22.83	30.11	42.34	26.35	34.96	80.40	29.82	50.89	73.56
70	.....	.....	50.34	.....	.....	89.36	.....	.....	74.04
75	25.67	33.33	.....	29.23	42.52	.....	36.37	58.24	.....
80	.....	.....	59.05	.....	.....	92.73	.....	.....	.....
90	28.09	37.28	64.27	32.31	50.41	94.68	40.00	69.59	.....
100	.....	.....	68.69	.....	.....	96.25	.....	.....	.....
105	30.58	45.34	.....	35.91	64.76	.....	43.37	68.10	.....
120	33.00	51.61	.....	38.17	73.96	.....	45.66	70.34	.....
135	.....	57.21	.....	.....	79.48	.....	48.85	72.58	.....
150	36.81	62.01	.....	43.89	82.99	.....	51.86	.....	.....
165	.....	67.16	.....	.....	.....	.....	54.07	.....	.....
180	40.79	70.74	.....	48.94	.....	.....	.....	.....	.....
210	44.92	.....	.....	55.10	.....	.....	.....	.....	.....
225	.....	.....	.....	.....	.....	.....	61.59	.....	.....
240	49.40	.....	.....	66.87	.....	.....	.....	.....	.....
270	54.42	.....	.....	68.22	.....	.....	.....	.....	.....
300	60.19	.....	.....	72.60	.....	.....	.....	.....	.....
330	66.11	.....	.....	.....	.....	.....	.....	.....	.....
345	.....	.....	.....	.....	.....	.....	69.91	.....	.....
360	71.87	.....	.....	.....	.....	.....	.....	.....	.....
390	73.67	.....	.....	.....	.....	.....	.....	.....	.....

five experiments at 5° C. In the last column is shown the percentage of the averages of intake. This percentage of the averages must not be confused with the average of the percentages, which would give a slightly different set of figures. In analyzing these variable groups we have used the percentage of the averages in attempting to construct a mathematical curve that would follow

the data. Similar groups of data for 20° and 35° C. are shown in tables IV and V.

The absorption data for the split peas were found on examination to be very difficult to analyze, owing to changes in rate of absorption, due almost certainly to internal physical changes in the seed. No attempt was made to carry out the work in so detailed a manner as in the case of *Xanthium* seeds. Enough has been done, however, to make it worth while to put the data on record. The results with three named varieties of peas at the three chosen temperatures are given in table VI.

Mathematical discussion

For purposes of mathematical discussion it is not considered essential to plot any curves of the data in addition to those given in fig. 1. Only such curves are used as are necessary to an understanding of the discussion. Anyone desiring the curves can easily plot them from the data.

In view of the fact that BROWN and WORLEY considered the curves of water absorption in *Hordeum* seeds as paraboloid running out toward a common asymptote, attention was turned first to the type of curve which would most nearly fit the data shown in the preceding tables. Even a casual examination of the data of tables I and II shows that the curves are not simple ones. Since the situation is somewhat simpler in the case of *Xanthium* seeds than in the split peas, the data from the former will be considered first.

XANTHIUM SEEDS

During the first moments of absorption (40–60 seconds) the entrance of water is exceedingly rapid; but in a short time the rate breaks sharply to a lower rate, which then decreases slowly but rather steadily during the main part of absorption, until approaching saturation begins to affect the rapidity of intake. In *Xanthium* seeds saturation occurs at about 50 per cent, and the final break in the curve caused by approaching saturation manifests itself at about 35–40 per cent, as is shown in the figures. The whole curve is thus apparently a composite curve made

up of at least three component curves. The general relations of these to one another in the composite curve are shown graphically in fig. 2, which has been somewhat exaggerated, especially in respect of the first curve, for the sake of clearness. The effect of the initial rapid intake is to throw the main part of the curve upward from the base line. Careful examination showed that it was not possible to find a parabolic curve that would follow the data at any temperature. The problem then was to find an empirical formula or equation or such a combination of equations as would very closely approximate the given data of observation. This was necessary

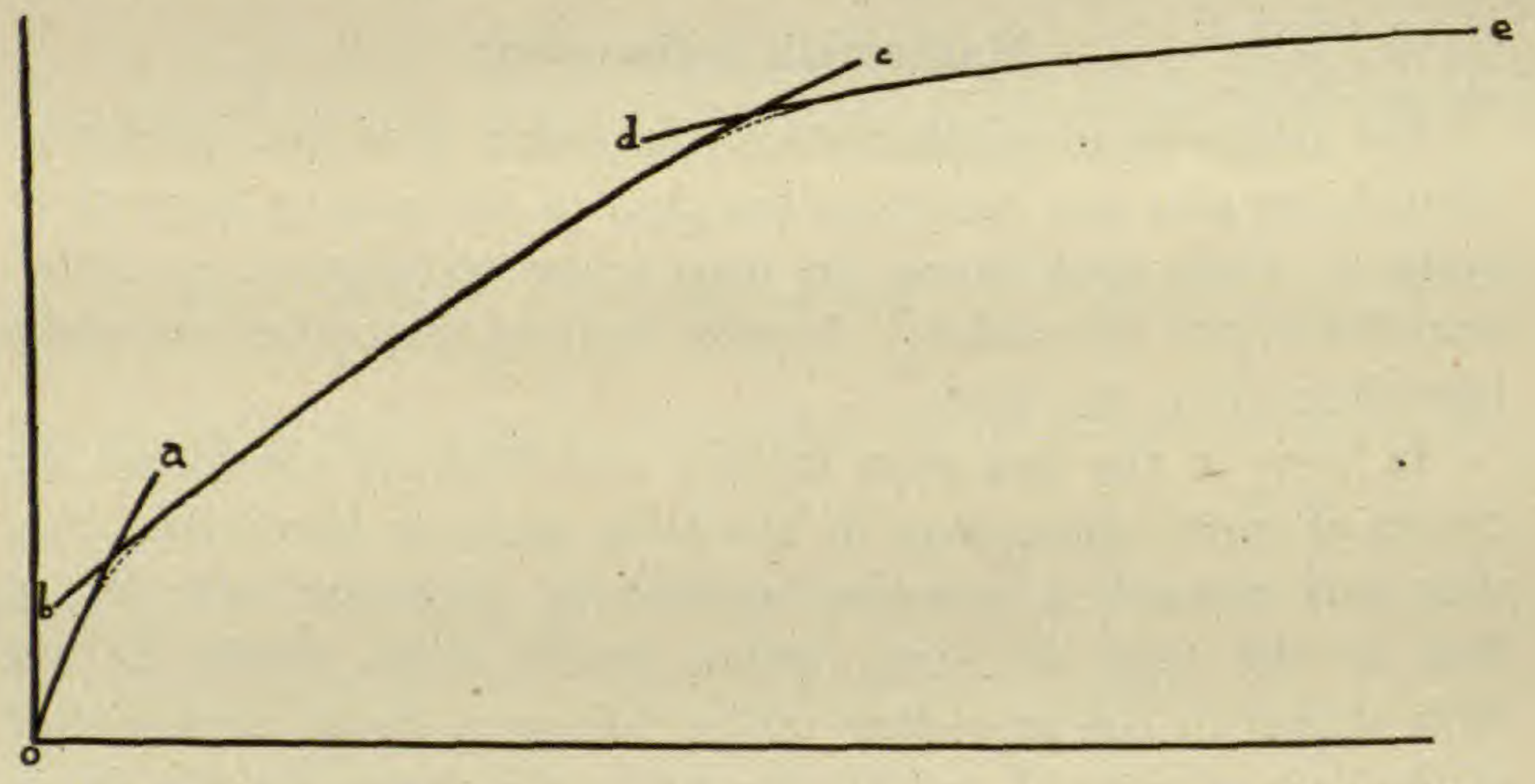

FIG. 2.—Curves showing composite nature of moisture intake curves in *Xanthium:* first curve exaggerated; *oa*, initial intake; *bc*, main curve; *de*, approaching saturation.

in order that precise and accurate methods of measuring tangents could be substituted for the uncertainties of the graphic method used by BROWN and WORLEY. The only proof we have that any equation or group of equations is adapted to such use lies in a comparison of the experimental data with corresponding values computed from the equation under consideration. As it is impossible to avoid slight irregularities in obtaining data, the equation must be so determined as to distribute the irregularities rather evenly on either side of the curve, as one would expect from the laws of chance variation.

Early in the investigation an equation was discovered which could be made to approximate very closely the series of data

obtained by measuring the total increase in weight due to absorption for different periods of immersion. This equation takes the form $y = a \log_{10}(bx+1)+c$, in which y = the total percentage of intake, and x = the length of time of immersion, a, b, and c being constants. In the later work it was found that a still closer approximation could be obtained by the employment of two equations of this form tangent to each other, the first equation representing the

TABLE VII

ALGEBRAIC CURVE FOR ABSORPTION DATA AT 5° C.; ABSORPTION IN PERCENTAGE OF AIR-DRY WEIGHT (*Xanthium*)

Time	Data low	Computed	Data high
1 minute	.....	1.055	1.124
15 minutes	.....	3.739	3.814
30 minutes	6.226	6.279	.....
45 minutes	8.544	8.545	.....
60 minutes	.....	10.591	10.747
75 minutes	.....	12.456	12.521
90 minutes	.....	14.169	14.202
105 minutes	15.710	15.753	.....
120 minutes	17.101	17.226	.....
135 minutes	.....	18.603	18.724
150 minutes	19.810*	19.896	.....
165 minutes	20.900*	21.113	.....
180 minutes	.....	22.265	22.270*
195 minutes	.....	23.356	23.400*
210 minutes	.....	24.394	24.540*
240 minutes	.....	26.327	26.701
270 minutes	27.965	28.098	.....
300 minutes	29.182	29.732	.....
330 minutes	.....	31.247	31.304
390 minutes	33.159	33.987	.....
450 minutes	35.072	36.410	.....
510 minutes	36.486	38.583	.....
570 minutes	38.400	40.524	.....
18.5 hours	45.020	52.975	.....

*Data from series II.

earlier data, the second representing the later data beyond the point of tangency. In one case it was found advantageous to introduce a third equation of this kind. The closeness with which this equation can be made to approximate the experimental data is truly surprising. It has been applied to the data furnished by BROWN and WORLEY for barley seeds, and approximates their data more closely than the calculated values they obtained from their formula. It must not be supposed, however, that the formula

can be successfully applied to all cases of absorption, or that it has any special significance beyond its applicability to measuring tangents accurately in all curves to which it fits.

In dealing with the data of table I it was found desirable to partially combine the two series at 5° C. because of irregularities in each set. As the seeds used in these early tests were not reduced to water temperature before immersion, some tests were run for

TABLE VIII

ALGEBRAIC CURVES FOR ABSORPTION DATA; INTAKE IN PERCENTAGE OF AIR-DRY WEIGHT (*Xanthium*)

TIME	20° C			TIME	35° C		
	Data low	Computed	Data high		Data low	Computed	Data high
1 minute . .	1.73	1.821	. . . . .	1 minute . .	2.45	2.840	. . . . .
15 minutes . .	. . . . .	6.481	6.806	15 minutes . .	. . . . .	10.136	10.89
30 minutes . .	. . . . .	10.599	11.00	30 minutes . .	16.41	16.471	. . . . .
45 minutes . .	. . . . .	14.213	14.55	45 minutes . .	. . . . .	21.720	21.81
60 minutes . .	17.38	17.396	. . . . .	60 minutes . .	. . . . .	26.317	26.38
75 minutes . .	20.20	20.239	. . . . .	75 minutes . .	30.21	30.305	. . . . .
90 minutes . .	. . . . .	22.809	22.81	90 minutes . .	. . . . .	33.855	33.89
105 minutes . .	25.12	25.153	. . . . .	105 minutes . .	. . . . .	37.054	37.11
120 minutes . .	. . . . .	27.308	27.44	120 minutes . .	39.80	39.964	. . . . .
135 minutes . .	. . . . .	29.301	29.32	135 minutes . .	41.87	42.635	. . . . .
150 minutes . .	31.06	31.157	. . . . .	150 minutes . .	43.25	45.102	. . . . .
165 minutes . .	32.80	32.891	. . . . .	180 minutes . .	45.24	49.534	. . . . .
180 minutes . .	. . . . .	34.520	34.54	7 hours	48.46	72.348	. . . . .
195 minutes . .	. . . . .	36.056	36.13				
210 minutes . .	37.43	37.507	. . . . .				
225 minutes . .	38.52	38.884	. . . . .				
240 minutes . .	39.39	40.194	. . . . .				
270 minutes . .	40.98	42.634	. . . . .				
300 minutes . .	42.57	44.871	. . . . .				
330 minutes . .	43.95	46.934	. . . . .				
360 minutes . .	44.75	48.849	. . . . .				
26 hours	47.28	84.280	. . . . .				

corrections of initial intake with seeds at water temperature. The result was a slight lowering of the initial intake at 5° C., and an increase at 35° C. These corrections were taken into consideration in deriving the values of the constants for computing the theoretical intake from the formula.

In the 5° C. curve the values for the constants a, b, and c in the equation given are as follows: $y=48.5 \log_{10} (0.098x+1)+0.85$. The closeness of the intake computed from this equation to the actual data is illustrated in tables VII and VIII.

The computed intake agrees very well with the experimental data until the absorption reaches 33 per cent, and from that on the data fall more and more below the computed values. This falling off of the actual intake marks the beginning of the effects of approaching saturation. It is evident that tangents to the curve may safely be computed up to about 35 per cent of intake, but beyond that point the tangents could not be used for comparisons of the rate of intake in different curves.

For the absorption at 20° C. the substituted values for the constants make the equation read $y = 61.5 \log_{10}(0.0136x + 1) + 1.46$, and the corresponding equation for 35° C. is $y = 74.5 \log_{10}(0.0184x + 1) + 2.25$. The closeness of the computed intake to the data of observation in each case is shown in table VIII.

In the 20° curve the effects of approaching saturation first manifest themselves at about 37.5 per cent, and in the 35° curve at about 40 per cent of intake. In each curve the computed values are strikingly close to the actual data. The uniformity of absorption and the agreement of the calculated intake to that observed has been a surprising feature of the work; and since the final break due to approaching saturation is always at or beyond 35 per cent, I have felt confident of accuracy in measuring tangents of the curves to that point.

In the later work the data could not be so satisfactorily represented by means of a single equation. By the use of two or three successive equations, however, each joined to its successor in a point of equal tangency, a very close agreement between calculated intake and experimental data was obtained. For the purpose of calculating tangents, and rates of intake, this composite curve is just as satisfactory as if it were developed from a single equation.

The 5° curve will be considered first. The three empirical equations used are as follows:

$$(1)\quad y = 14.3 \log_{10}(0.078x + 1) + 1.398$$
$$(2)\quad y = 35.07 \log_{10}(0.0121x + 1) + 4.195$$
$$(3)\quad y = 87.95 \log_{10}(0.0023x + 1) + 8.625$$

The first two curves have equal tangents for $x = 35.35$, and the last two for $x = 150.89$ (minutes). The breaks in the curve

are very small. Thus, at the first break, in curve 1, $y=9.605814$; while in curve 2, $y=9.6056$ at the common point of tangency with curve 1. At this common point the two curves are only 0.000214 (per cent) apart. Similarly at the second break, for curve 2, $y=20.01634$, and for curve 3, $y=20.016284$, a break of only 0.000056 per cent. This combination curve runs remarkably close to the data of observation and gives perhaps the best series presented. The calculated and observed intake is shown in table IX. Data in last column, table III.

TABLE IX

ALGEBRAIC CURVES FOR ABSORPTION DATA; INTAKE IN PERCENTAGE OF AIR-DRY WEIGHT (*Xanthium*), 5° C.

Time (minutes)	Data low	Computed	Data high
1	1.64	1.86	.....
5	.....	3.44	.....
10	4.97	4.98	.....
15	.....	6.21	6.22
30	8.77	8.89	.....
45	.....	10.82	.....
60	12.50	12.51	.....
90	.....	15.42	15.43
120	17.77	17.86	.....
150	.....	19.96	19.99
180	.....	21.86	.....
240	.....	25.41	25.42
300	.....	28.67	.....
360	.....	31.67	.....
420	.....	34.46	34.55
540	38.56	39.46	.....

The data obtained with *Xanthium* seeds at 20 and 35° C. were given similar treatment. Two equations were used for the 20° data as follows:

$$(1)\quad y=23.77\log_{10}(0.088x+1)+1.524$$
$$(2)\quad y=57.13\log_{10}(0.0132x+1)+6.616$$

These two curves have tangents equal for $x=34.52$, at which point curve 1 has $y=15.931972$, and curve 2, $y=15.931732$, only 0.00024 per cent apart.

In the 35° data, also, two successive equations were used:

$$(1)\quad y=34.92\log_{10}(0.0983x+1)+1.40$$
$$(2)\quad y=73.05\log_{10}(0.0286x+1)+6.53$$

The point of equal tangency in these curves comes at $x=22.91$, and at this point in curve 1, $y=19.28409$, while in curve 2, $y=19.28407$. The break therefore is only 0.00002 per cent. The agreement between computed and observed intake here is not quite so close as in the 5° curve, but is still very good (see table X, the data for which come from the final columns of tables IV and V).

It is apparent in these later results, just as in the earlier ones, that approaching saturation does not begin to interfere with absorption rates until 35–40 per cent of intake has occurred. It should be quite clear, also, that the equations employed run so

TABLE X

ALGEBRAIC CURVES FOR ABSORPTION DATA; INTAKE IN PERCENTAGE OF AIR-DRY WEIGHT (*Xanthium*)

TIME (MINUTES)	20°			TIME (MINUTES)	35°		
	Data low	Computed	Data high		Data low	Computed	Data high
1	2.38	2.39	.....	1	.....	2.82	2.83
5	.....	5.29	5.34	5	.....	7.46	.....
10	7.92	8.05	.....	10	.....	11.78	11.82
15	.....	10.21	10.32	15	15.11	15.14	.....
30	14.64	14.86	.....	20	17.84	17.89	.....
45	.....	18.18	18.54	30	.....	22.38	22.45
60	.....	21.09	21.13	40	.....	26.29	26.32
75	23.64	23.69	.....	50	29.70	29.76	.....
90	.....	26.04	.....	60	.....	32.90	32.95
105	28.17	28.19	.....	70	35.71	35.75	.....
120	30.14	30.17	.....	80	38.13	38.37	.....
135	.....	32.00	32.18	90	40.19	40.79	.....
150	.....	33.71	33.72	100	41.78	43.03	.....
165	35.20	35.30	.....				
180	36.66	36.80	.....				

close to the observed data that the velocity of intake can be measured at any given moment with great accuracy. Instead of plotting curves and attempting to measure the tangents graphically, they have been calculated from the known formula.

The velocity of intake has been computed from the tangents for six points on each temperature curve of intake. These points coincide with those chosen by BROWN and WORLEY, as follows: 5, 7.5, 10, 15, 20, and 25 per cent of intake. The percentage hourly rate of intake for these points, on each curve shown in tables VII and VIII, together with the logarithms of the hourly rates of intake, are shown in table XI.

TABLE XI

WATER INTAKE IN *Xanthium* SEEDS

INTAKE PERCENTAGE	5°		20°		35°	
	Velocity in percentage per hour	Logarithm	Velocity in percentage per hour	Logarithm	Velocity in percentage per hour	Logarithm
$y=5$.....	10.1705	1.007342	19.0894	1.280792	32.8097	1.516002
$y=7.5$.....	9.0322	0.955794	17.3838	1.240145	30.3700	1.482445
$y=10$.....	8.0214	0.904250	15.8304	1.199492	28.1116	1.448886
$y=15$.....	6.3264	0.801157	13.1278	1.118191	24.0865	1.381774
$y=20$.....	4.9896	0.698066	10.8866	1.036893	20.6376	1.314659
$y=25$.....	3.9351	0.594956	9.0280	0.955592	17.6826	1.247546

In the later work the velocity calculated from the tangents is expressed in percentage per minute, instead of percentage per hour. The velocities for the same six points, on the curves shown in tables IX and X, are given in table XII.

TABLE XII

WATER INTAKE IN *Xanthium* SEEDS

INTAKE PERCENTAGE	5°		20°		35°	
	Velocity in percentage per minute	$\log_{10}$ Velocity	Velocity in percentage per minute	$\log_{10}$ Velocity	Velocity in percentage per minute	$\log_{10}$ Velocity
$y=5$.....	0.27122	$\bar{1}.433322$	0.64872	$\bar{1}.812057$	1.17576	0.070318
$y=7.5$.....	0.18134	$\bar{1}.258494$	0.50920	$\bar{1}.706888$	0.99707	$\bar{1}.998726$
$y=10$.....	0.12589	$\bar{1}.099992$	0.39968	$\bar{1}.601702$	0.84554	$\bar{1}.927134$
$y=15$.....	0.09066	$\bar{2}.957416$	0.24624	$\bar{1}.391358$	0.60806	$\bar{1}.783947$
$y=20$.....	0.06537	$\bar{2}.815378$	0.19096	$\bar{1}.280943$	0.44820	$\bar{1}.651472$
$y=25$.....	0.05722	$\bar{2}.757548$	0.15611	$\bar{1}.193431$	0.38284	$\bar{1}.583018$

TEMPERATURE COEFFICIENT.—Having now obtained the rate of intake at chosen points on each curve, we can proceed to determine the quantitative effects of temperature on the rate of moisture intake. First we must know the ratio of the velocity at 20° to that of 5° C., and of the velocity at 35° to that at 20° C. These ratios for the intake velocities presented in tables XI and XII are given in table XIII.

In the earlier data, represented by table XI, if we take the average velocity at 5° C. as unity, we have the comparative mean velocities at 20 and 35° C. according to the ratio $1:2.05:2.05\times 1.83=3.75$. Since the temperature of intake in the last curve

is 30° higher than the first, the mean value of Q_{10} will be obtained by extracting the cube root of the final term, 3.75, which is 1.55.

In the later data, table XII, the mean value of Q_{10} is higher. The final term of the ratio is 6.11, and its cube root 1.83. In both cases the value falls between the coefficient of temperature effects on physical and on chemical processes, but in the last case it approaches the van't Hoff coefficient. These figures are comparable with the value of Q_{10} obtained by BROWN and WORLEY for barley, as they have been obtained in exactly the same manner. The value of the temperature coefficient for *Hordeum* was 2.02.

BROWN and WORLEY considered that the velocity of intake was almost exactly an exponential function of the temperature. If it is,

TABLE XIII

RATIOS OF INTAKE VELOCITIES (*Xanthium*)

INTAKE PERCENTAGE	DATA TABLE XI		DATA TABLE XII	
	Velocity 20° / Velocity 5°	Velocity 35° / Velocity 20°	Velocity 20° / Velocity 5°	Velocity 35° / Velocity 20°
$y=$ 5.0.....	1.88	1.72	2.39	1.81
$y=$ 7.5.....	1.92	1.75	2.81	1.96
$y=$10.0.....	1.97	1.80	3.17	2.12
$y=$15.0.....	2.08	1.83	2.71	2.47
$y=$20.0.....	2.18	1.90	2.92	2.35
$y=$25.0.....	2.29	1.96	2.73	2.45
Mean ratios	2.05	1.83	2.79	2.19

logarithms of the velocities plotted against the temperature must lie in straight lines. They show in their second diagram such a plot of the logarithms, and state that the course of the lines in the diagram, in respect both of the straightness and of the agreement of inclination, furnishes evidence of a most conclusive character that the rate at which water is absorbed by barley seeds is an exponential function of the temperature. They call attention to the rarity with which physical properties show an exponential increase with rise in temperature, and then propose that the change is chemical and probably involves a simplification of the water molecule, as already stated.

The logarithms of the velocity of water intake by *Xanthium* seeds have been plotted similarly in fig. 3. The curves plotted

above the zero line represent the velocities for the earlier *Xanthium* data of table XI, while those below the zero line are from the later data from table XII. These curves will be discussed later.

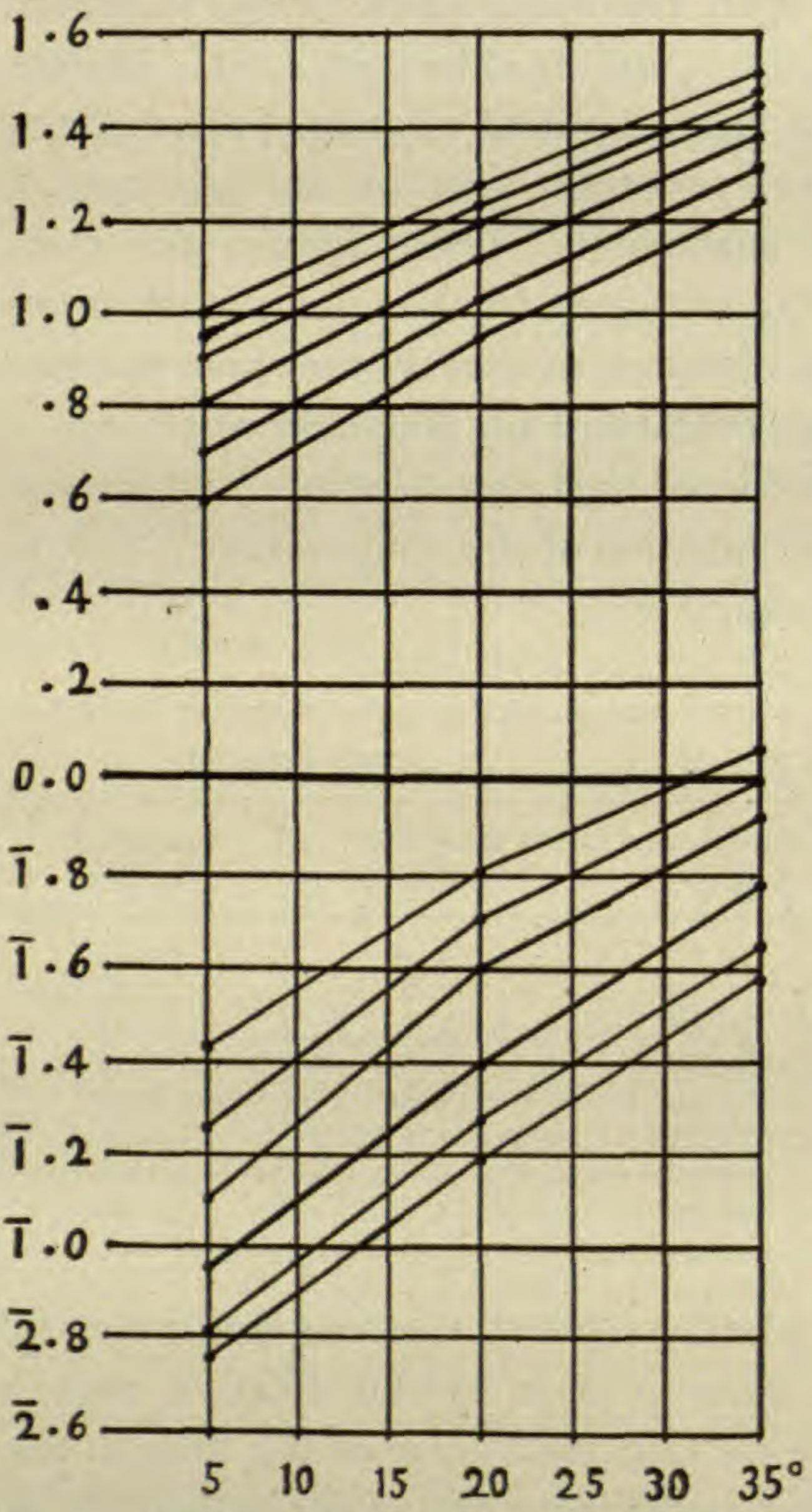

FIG. 3.—Logarithms of velocity plotted against temperature: upper series plotted from table XI, lower series from table XII; *Xanthium* seeds.

SPLIT PEAS

The split peas offered special difficulties from the mathematical side, and no attempt is made to present a complete account of the analysis of the data given in table VI. The variability of the data is much greater than in the case of cockleburs. The absorption is fairly consistent during the first hour, or, at high temperatures, during the first 15 or 20 minutes. After a certain critical percentage has been reached, however, they show a remarkable rise above the ideal curve indicated by the first part of the absorption. This critical percentage is about 20 per cent in the case of the Tom Thumb variety, about 23 per cent in the Canada Green field pea, and about 30 per cent for the Small Scotch Yellow commercial. The reasons for the rise in the rate of absorption will be considered in the general discussion.

As the Small Scotch Yellow gives us the longest period of consistent intake I shall present here data for this variety only,

and only for that portion of the curves which precedes the rise in rate.

Difficulties were encountered in choosing an empirical formula for the split pea data, owing partly no doubt to the fact that no duplicate tests were run, and the only set of data showed rather large irregularities at the beginning of the absorption. Curves closely approximating the data beyond 5 minutes ran below the point of origin. The one minute value ran quite too high in the 20 and 35° C. data, and somewhat too low in the 5° C. series. In any case the constant c in the formula was so small that it was thought best, after considering all possibilities, to run the computed curves through the point of origin, and omit that constant altogether. The generalized formula then takes the form $y = a \log_{10} (bx+1)$.

TABLE XIV

ALGEBRAIC CURVE FOR ABSORPTION DATA; SMALL SCOTCH YELLOW SPLIT PEA

TIME (MINUTES)	5°			20°			35°		
	Low	Computed	High	Low	Computed	High	Low	Computed	High
1	1.77	1.81	.....	.....	3.75	5.38	.....	4.20	5.77
5	.....	7.25	7.26	13.26	13.27	.....	16.34	16.41	.....
10	11.50	11.88	.....	19.98	20.21	.....	.....	26.47	26.54
15	.....	15.30	15.58	.....	24.93	25.09	33.65	33.73	.....
20	.....	.....	.....	.....	.....	.....	.....	39.43	41.54
30	.....	22.16	22.48	33.69	33.84	.....	.....	.....	.....
45	.....	26.64	.....	.....	.....	.....	.....	Break up	.....
48	.....	.....	.....	.....	40.30	43.01	.....	.....	.....
60	29.82	29.97	.....	.....	Break up	.....	.....	.....	.....
75	.....	32.62 Break up	36.37	.....	.....	.....	.....	.....	.....

The three formulae, for the 5, 20, and 35° C. curves for the Small Scotch Yellow peas, with values of a and b substituted, are as follows:

$$5° \text{ C.}: y = 30.13 \log_{10} (0.148x+1)$$
$$20° \text{ C.}: y = 34.58 \log_{10} (0.284x+1)$$
$$35° \text{ C.}: y = 60.90 \log_{10} (0.172x+1)$$

Using these empirical formulae, we have secured a fair agreement between calculated and observed intake, not so close as in the case of *Xanthium*, but much closer than is frequently obtained

in attempts to reduce biological phenomena to mathematical expressions (see table XIV).

The velocity of intake at the same six percentages used for the *Xanthium* seeds has been calculated from the tangents to the curves. The velocity in percentage per minute, and the logarithms of the velocities are shown in table XV.

TABLE XV

WATER INTAKE IN SMALL SCOTCH YELLOW SPLIT PEA

INTAKE PERCENTAGE	5°		20°		35°	
	Velocity in percentage per minute	$\log_{10}$ velocity	Velocity in percentage per minute	$\log_{10}$ velocity	Velocity in percentage per minute	$\log_{10}$ velocity
$y=5$	1.32160	0.121100	3.05728	0.485335	3.76544	0.575827
$y=7.5$	1.09175	0.038122	2.58846	0.413041	3.42593	0.534779
$y=10$	0.90188	$\bar{1}$.955148	2.19152	0.340745	3.11692	0.493725
$y=15$	0.61546	$\bar{1}$.789200	1.57091	0.196151	2.58002	0.411623
$y=20$	0.42000	$\bar{1}$.623249	1.12605	0.051558	2.13562	0.329524
$y=25$	0.28662	$\bar{1}$.457306	0.80717	$\bar{1}$.906965	1.76775	0.247421

The ratios of the intake velocities for the split peas were obtained from the data of table XV, and are presented in table XVI.

TABLE XVI

RATIOS OF INTAKE VELOCITIES; SMALL SCOTCH YELLOW SPLIT PEAS

Intake percentage	$\frac{\text{Velocity } 20°}{\text{Velocity } 5°}$	$\frac{\text{Velocity } 35°}{\text{Velocity } 20°}$
$y=5$	2.31	1.23
$y=7.5$	2.37	1.32
$y=10$	2.43	1.42
$y=15$	2.55	1.64
$y=20$	2.68	1.90
$y=25$	2.82	2.19
Mean ratio	2.53	1.62

From the mean ratios we find that the value of Q_{10} in this case is 1.6, or just a little higher than the earlier determination for *Xanthium*. Since the calculations in the case of split peas are made from single equation curves, all passing through the point of

origin, they offer the best possible opportunity to study the question of straight line plots of logarithms against temperature. These are shown in fig. 4. It is seen that they are decidedly not straight lines.

Having now presented in some detail the results of the mathematical analysis of the data, which has been carried out in such a way as to make possible a comparison between this work and that of BROWN and WORLEY, I shall discuss briefly the significance of the results.

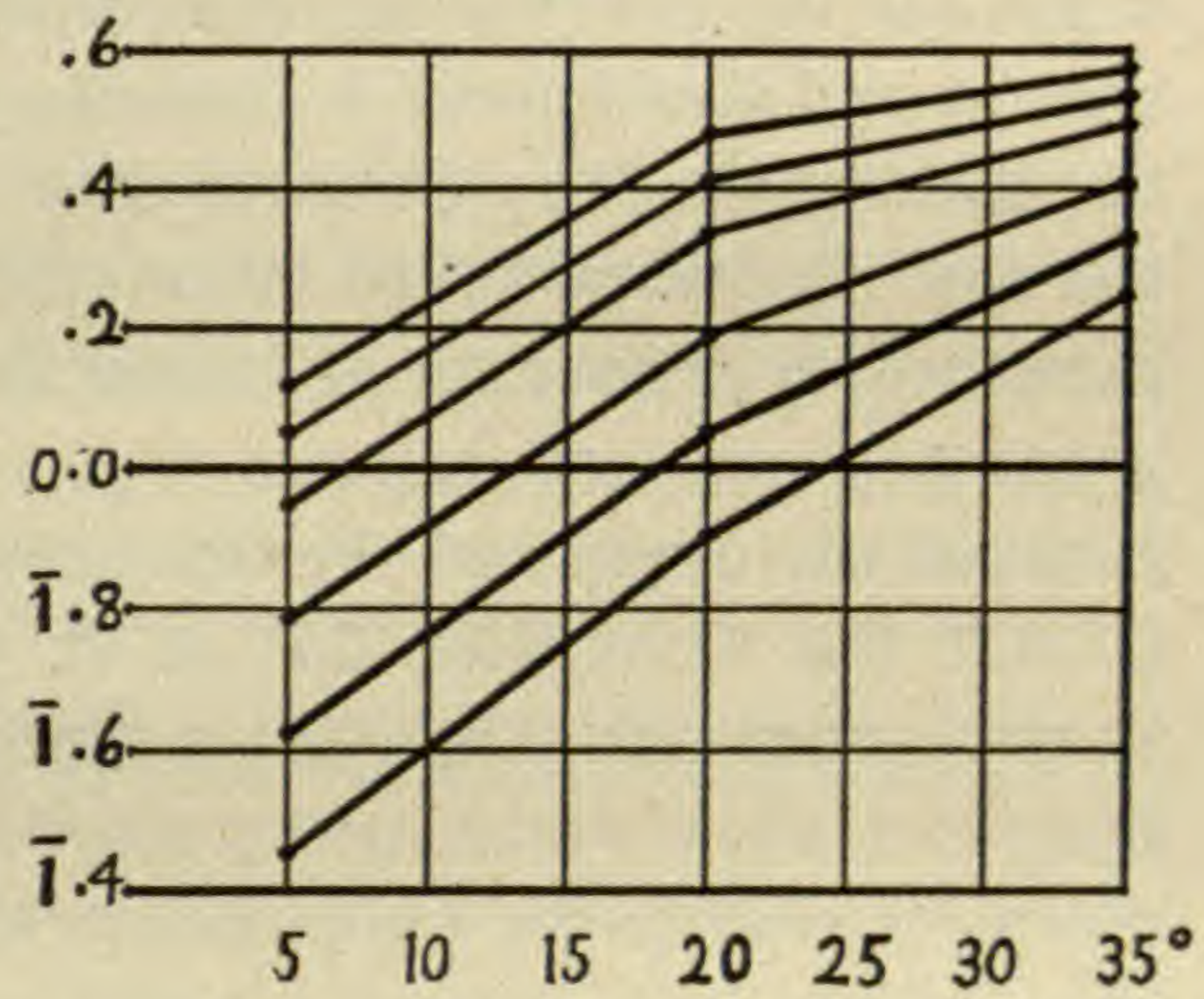

FIG. 4.—Logarithms of velocity plotted against temperature, split peas, table XV.

Discussion

There are several features of the work by BROWN and WORLEY which need to be considered in judging its value. Attention was called in the introduction to the rather rough method of securing tangents, which, however, was quite skilfully used. In view of the fact that the early phases of absorption were not studied by them, however, it is possible that the tangents they obtained between the point of origin and the first intake data at each temperature would not agree with those of a curve plotted at close intervals. If the barley seeds were to show a large initial intake, the curve would be thrown upward from the base, and the succeeding portion of the curve would have a different course, affecting the very portion of the curve where the tangents are measured in determining intake velocities. It is this early part of the curve which is important, for the tangents are measured for that part of the curve between the origin and 25 per cent of intake.

The greatest disadvantage in the data supplied by BROWN and WORLEY is the long time interval between observations, and especially the long first interval. Their first observations were taken at 5–6 hours after the beginning of absorption. If I had waited 5 hours for the first observations in any of the work presented

in this paper, all of the tangents used in measuring intake velocities would have fallen on that part of the curve between the point of origin and the first reading, all of which is constructed from imagination, as an "ideal curve." In the case of barley it is not so serious, but it is only in the 3.8° curve that all of the tangents fall beyond the first observation. In their 21.1° curve the first observation showed over 9 per cent of intake, from which it is seen that the 5 and 7.5 per cent tangents were measured on a "guess curve" between the origin and the first observation, and the 34.6° curve is still less favorable; for in it the first observation shows nearly 17 per cent of intake, so that 4 out of 6 tangents used were measured on a curve constructed entirely without data. This matter is vital to the whole theory they propose, for they had but three points in plotting logarithms of velocities against temperatures, and if one of the points is insecure no conclusions can be drawn. The other two points are bound to be in a straight line. In four cases out of six, the third point is not established by data, and in two of the plotted logarithm-temperature curves, both the second and the third points are derived from tangents whose determination is insecure. The evidence offered, therefore, that the velocity of intake is an exponential function of the temperature, is not very convincing. In this work I have used short time intervals to understand better the curve whose tangents were to be measured. Our short intervals have the disadvantage that water movement goes on in the seed during weighing which occurs frequently. There is no intake during weighing, of course, but distribution of water already taken in continues. I have felt that the advantages of the close intervals between weighings exceed by far any disadvantage that might exist.

In the case of *Xanthium*, with a semipermeable coat, and in split peas without the coat, I have found that the plotting of logarithms of velocity against temperatures does not yield straight lines. The nearest approach to straight lines is seen in the upper half of fig. 3, but even here there is a slight divergence, always in the same direction. A somewhat greater divergence from straight lines is seen in the lower half of fig. 3, and a very marked divergence is seen in fig. 4, in the case of split peas. From the data I conclude

that plotting logs of velocities against temperatures will yield some kind of a curve, but there are not enough data at hand to determine anything as to the character of the curve. The general conclusion to be drawn from this part of the work is that the evidence, as far as it goes, is rather against the assumption that the velocity of intake is an exponential function of the temperature.

Another point that deserves notice is the nature of the curves of water intake. BROWN and WORLEY called their curves paraboloid and described them as running out toward a common asymptote. The language, of course, must have been intended in a very loose sense, for parabolic curves passing through a common point of origin, as theirs do, could never have a common asymptote. It was found impossible to fit a parabolic formula to the intake data presented, but from the figures given in tabular form (tables VII–X and XIV) it is evident that the logarithmic curve $y=a \log_{10}(bx+1)+c$ may be made to fit the data very closely. Furthermore I have taken the 3.8° barley data and attempted to fit to it both the logarithmic and a hyperbolic equation made to pass through the origin and the second and fourth values of their data. I have found that the logarithmic equation fits much closer to their data than the hyperbolic equation. The two sets of values and the original data are given for comparison. The time and data columns are from BROWN and WORLEY.

Time	Data	Computed (logarithmic)	Computed (hyperbolic)
5.58 hours	4.42	4.41	5.21
24.75 hours	11.82	11.82	11.82
48.83 hours	18.52	18.49	17.99
72.25 hours	23.42	23.43	23.42
96.50 hours	27.42	27.56	28.78
144.25 hours	34.02	33.89	38.99

The logarithmic equation used in this comparison is $y=48.6 \log_{10}(0.025x+1)$, and the hyperbolic equation, $y=0.2024 \sqrt{x^2+112.988x}$.

Considering the closeness of agreement which is obtainable with the logarithmic formula, it seems more reasonable to consider the curves of water intake, even in the case of barley seeds, as logarithmic rather than hyperbolic.

If the velocity of absorption were an exponential function of the temperature, the relation between temperature and the rate of entry of water into the seeds might be expressed by an equation of the form $v = ae^{k\theta}$ in which θ is the temperature. As I have obtained evidence somewhat adverse to the assumption that velocity of absorption is an exponential function of the temperature, this equation does not hold. Wherever the logarithmic formula $y = a \log_{10}(bx+1)+c$ holds for the curves of absorption, the velocity of intake may be represented by the formula $v = ae^{-k\phi}$ in which ϕ is the percentage of water already absorbed. In other words, the velocity of intake is approximately an inverse exponential function of the total preceding absorption. It is not claimed that this is true for all cases of absorption, but that it is just as true as the logarithmic equation used. Wherever that equation holds, the velocity formula holds.

The chief interest centers in the temperature coefficient of absorption. I have obtained coefficients ranging from 1.55 to 1.83 in *Xanthium* seeds, and 1.6 in split peas. These are all above the temperature coefficient of physical changes, and below that for chemical change. BROWN and WORLEY obtained a value above 2, and adopted the idea that absorption was conditioned as to rate, in the case they studied, by some chemical change. In seeking a chemical change to account for their observations, they suggested that the semipermeable seed coat of barley was involved in a special way, in its relation to complex or simplified water molecules. They suggested the possibility that the differential septum (semipermeable coat) permits only hydrone to penetrate it, and that the temperature rise increases the proportion of hydrone in solution. One of the main difficulties in the way of accepting such a hypothesis as to the relation of hydrone to semipermeable membranes, is its implication that all semipermeable membranes should behave alike. *Xanthium* and *Hordeum* both have semipermeable membranes, and if the rate of water passage depended solely on the proportion of hydrone, treatment of either seed should give the same results. It is a notable fact, however, that semipermeable membranes are always individualistic. Each kind has its own behavior, no two

kinds acting exactly alike. It would not be possible to accept without modification any theory which assumes that differential septa are alike in behavior. I do not mean to say that water is not simplified in structure as it is warmed, nor that such a change would not increase the rate of absorption, but it seems entirely possible to account for the high temperature coefficients found in absorption phenomena without the necessity of assuming such a change, or making it the sole change involved in the process. The substances of which the seeds are composed, membranes, embryo, and storage products, are all largely colloidal. These colloidal materials undoubtedly are modified in state of aggregation by being subjected during wetting to low or high temperatures. Higher temperatures usually increase dispersion and increase the water-holding capacity of organic colloids, and lower temperatures reverse the process. It does not seem possible that such changes could be absent during absorption, and they must go far to explain the differences in intake rates and the values of Q_{10}, which stand between those found for purely physical and purely chemical processes. Absorption is a complex process, probably involving both physical and chemical factors, and the values of Q_{10} may be considered the resultant of the effects of temperature on both classes of factors. The fact that we get about the same value for Q_{10} in absorption without a semipermeable coat as with such a coat indicates that the membrane is not necessarily the rate determining factor.

DENNY (**2**) has shown that membranes differ greatly in their power to transmit water. If the seed coat transmits water more slowly than seed substance can absorb it, the transmission rate is a limiting factor on the absorption rate. If the transmission power of the coat exceeds the absorption power of the seed substance, however, the latter determines the rate. Again, if seed coat, embryo, and endosperm form a very non-homogeneous structure, the absorption rate may be dominated first by one of the structures, and later by the others in succession, giving peculiar absorption curves, difficult to analyze mathematically.

It was noted that *Xanthium* seeds showed a very rapid initial intake during a minute or less, after which the rate broke sharply to a

lower rate. Two explanations suggest themselves for this. The coat may absorb water more readily than seed substance, and the initial intake may represent the saturation of the seed coat, or the rapid initial intake may be caused by the fact that at first the absorbing substance and water are in direct contact, but after a short time the water absorbed by the interior of the seed must penetrate a layer of saturated substance before it can reach the actively absorbing material. This outer saturated layer may offer resistance to intake in the form of friction with the moving water. As this layer becomes thicker and thicker all the time, it may tend more and more to reduce the absorption rate. Changes in the velocity of absorption due to such causes might be found in any case of water intake.

Finally, something should be said about the rise in the intake rate in split peas after a certain critical percentage of intake has been reached. During absorption one can observe that the hemispherical cotyledons become swollen first around the thin edge where water is penetrating from both sides. Looking at the flat side of the cotyledon, one can see that the edge has become raised up, while the center remains as it was originally, and appears depressed. The flat side has become concave. It seems evident that a band of dry material extends across the middle of the cotyledon from the center of the spherical side to the center of the flat side, and that imbibition forces at work in the edge of the seed are pulling at this dry band. After the critical intake has been reached, the center of the flat side soon swells out, and the concavity disappears. It is practically certain that the seed substance actually cracks apart during this process, leaving interior cavities that fill up with water. This idea is strongly supported by unpublished data, collected by DUDLEY J. PRATT, who worked in the laboratory of the University of Kansas, on the effects of acids and bases on the swelling of pea cotyledons. He was able to detect clearly the formation of such cavities during absorption, and some of them are of considerable size, as when strong hydrates or acids cause excessive swelling. This breaking up of the internal tissues of the cotyledon satisfactorily accounts for the peculiarities observed in absorption curves in split peas.

It is my conviction, after a number of years of experience with absorption phenomena, that absorption is a complex process dependent on a number of factors, some of which may be external, but many of which are internal. I have become convinced that we should not expect a single formula or rate law to apply to absorption in general. Each case of absorption is likely to present a problem in itself, and to differ, slightly at least, from any other case, because of both qualitative and quantitative differences in the numerous factors determining absorption rates.

Summary

1. This paper deals with the quantitative influence of temperature on the velocity of moisture intake by certain seeds, chosen for the presence and absence of semipermeable coats. *Xanthium pennsylvanicum* Wallr. and commercial and garden peas were used, the latter with coats removed.

2. The curves of water intake were found to be complex, but can be represented by a logarithmic equation or series of equations of the form $y = a \log_{10}(bx+1)+c$.

3. The analysis of the data presented does not support the theory of BROWN and WORLEY that the velocity of intake is an exponential function of the temperature, but the velocity of intake at any given moment in the seeds studied is approximately an inverse exponential function of the amount of water previously absorbed.

4. The mean value of Q_{10} in *Xanthium* seeds was in one instance 1.55, in another 1.83, and in split peas of the Small Scotch Yellow variety 1.6.

5. These values do not indicate that absorption is conditioned by some single chemical change like simplification of water to hydrone as the temperature rises, but are believed to indicate that absorption at different temperatures involves both physical and chemical changes.

6. The main chemical changes with rise of temperature are believed to occur in the colloids of the seed, and semipermeability, as such, is thought not to be an important factor in determining the rate of water absorption.

7. The paper considers critically the methods and interpretation of the similar work of BROWN and WORLEY on *Hordeum* seeds.

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PETIOLAR GLANDS IN THE PLUM[1]

M. J. Dorsey and Freeman Weiss

(with plates XX, XXI)

True functional glands are present in the plum in three positions: on the leaf serrations, on the leaf base, and on the petiole. In the peach, plum, and cherry, the petiolar glands have been given a place of considerable taxonomic importance. In the course of the fruit breeding work at the Minnesota Agricultural Experiment Station, excellent material became available for a study of the glands in the plum in certain hybrids and pure forms. Since certain questions regarding their variation and morphology appeared to be as yet open, the investigation reported herein was begun.

In a historical review of the taxonomic use of the petiolar glands in the stone fruits, Gregory (3) showed that the earlier writers had ignored these structures; while later pomologists had made use of them in distinguishing major groups, as in the peach. Other writers, however, questioned the taxonomic value of glands, because of the variation observed in number, shape, and position. From an extensive study of the leading varieties of the peach, Gregory concluded that on typical shoots the glands were constant, and that in many cases their shape could serve to separate groups of varieties. He arranged the better known peach varieties under three types of glands, reniform, globose, and indistinctive, but pointed out that mixed and transitional types occur.

Hedrick and others (4) record the gland condition on the petiole and leaf serrations in the descriptions of the principal varieties of plums in New York. Similar data have been brought together for cherries (Hedrick *et al.* 5) and peaches (Hedrick *et al.* 6). In the latter work the statement is made that "no one familiar with any considerable number of varieties of peaches

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would attach very great importance to glands in a system of classification."

On the whole, the tendency of later writers has been to attach less significance to glands in classification than has been done by earlier writers. In technical fruit descriptions, or in systematic classifications, it is evident that the value of a character as a distinguishing feature between forms depends largely upon its constancy of expression. Consequently, a statistical analysis was undertaken with the object of determining the number and disposition of glands in certain species and hybrids available.

Material

Data were first collected in 1914 in the F_1 generation of crosses between Burbank (*Prunus triflora*) and Wolf (*P. americana*), and Abundance (*P. triflora*) and Wolf. The gland condition was subsequently (August 1916) obtained in an additional number of species and interspecific hybrids. Single trees in each case of as nearly uniform age and size as possible were selected, and 400 leaves, on all trees which bore this number, were taken at random from vigorous 1-year shoots. By following this method of collection consistently on trees under fairly uniform growth conditions, the data obtained for the different forms are as nearly comparable as can be obtained under field culture.

There are a number of factors which influence gland development. In general it may be stated that those conditions which produce vigorous vegetative growth favor gland development, since on old trees or on trees subjected to unfavorable growth conditions, the petiolar glands become much reduced, sometimes even disappearing, although normally present in the varieties. On the other hand, position has an influence on glandular development. Leaves borne at the basal position on terminal growth, on fruit spurs or thorns and also in flower buds, typically bear no glands at all or have them less well developed than leaves borne at other points.

The arrangement of the glands (that is, whether opposite or alternate on the petiole or leaf) was not recorded. Glands occur both in pairs and alternately, near together or widely separated,

but since they vary independently on either side of the petiole, their relative position appears to be only incidental.

Variation in gland position and number

In horticultural literature, glands have been described with respect to color, type or shape, size, number, and position. In

TABLE I

SELECTED INSTANCES ILLUSTRATING METHOD OF RECORDING DATA AND SHOWING VARIABILITY OF GLANDS (A) ON DIFFERENT LEAVES WITHIN A VARIETY, (B) WITH REFERENCE TO POSITION ON PETIOLE OR LEAF BASE, (C) WITH REFERENCE TO DIFFERENT VARIETIES, AND (D) WITHIN SAME VARIETY DURING DIFFERENT SEASONS.

No. borne on		Abundance × Wolf no. 35		Burbank		Burbank × Wolf no. 9	
Petiole	Leaf	1914	1917	1914	1917	1914	1917
0	0	15	38	10	45	44	52
0	1	27	35	16	63	25	44
0	2	28	37	32	45	28	45
0	3		1				
1	0	34	40	6	8	25	33
1	1	29	33	8	29	63	48
1	2	1	16	9	25	9	6
1	3				1		
2	0	219	188	67	65	141	161
2	1	39	10	45	47	49	5
2	2	4		33	18	1	1
2	3				1		
3	0	11	2	55	20	8	4
3	1	3		33	17	6	
3	2			12			
4	0			33	7		
4	1	1		24	3	1	1
4	2			9	1		
5	0			2	2		
5	1			3	1		
5	2			1	1		
5	3				1		
6	0			2			
Glands on petiole		604	491	919	505	525	437
Glands on leaf		143	187	321	349	220	202

the plum the globose form is the prevailing type, and the true reniform type is found so seldom that little attention has been given to shape. The color of the mature glands in the plum is dark brown; and since these studies of number and position were made on mature leaves, color characters were also not recorded.

Data taken as to position and number were arranged in the form illustrated in table I, in which each leaf is classified with respect to the position and number of its glands. For instance, in Burbank 67 leaves bore two glands on the petiole and none on the leaf in 1914, and in 1917, 65 leaves fell in this class. A number of other varieties could have been included, but these were selected as typical of the great variability encountered.

Table I shows that in number and position glands are extremely variable on different leaves within a variety, but that the range of variability is fairly typical for each variety. The number of glands borne on the petiole is greater than the number borne on the leaf base, and while the number borne in each position is considerably different from season to season, yet the grouping opposite each class is quite similar in each variety in spite of the fact that the 1917 data were taken from different trees, but of the same clones, from those of 1914. Taking Burbank again as an illustration of variability, it will be seen that some leaves have no glands on either the petiole or leaf, while others bear as many as five on the petiole and three on the leaf. If observations as to gland condition made on a few leaves or herbarium specimens are considered from the standpoint of the variation shown, it will be evident that some caution must be exercised in classifying the gland condition.

Referring to the variability of glands within the species, it will be seen that a similar condition is found to that shown within varieties. A summary of the position and number of glands in all the species investigated is presented in table II, in which the gland condition is given for a total of 3477 leaves.

Four points are of interest in table II: (1) without exception there are more glands borne on the petiole than on the leaf base; (2) when there is one gland present it may be borne either on the leaf base or on the petiole; (3) when two glands are present, the larger number is without exception borne on the petiole; and (4) when more than two glands are present, without exception a strikingly larger number occur on the petiole.

For the convenience of the reader the data presented in table II, with the addition of data from certain interspecific hybrids,

TABLE II

SUMMARY OF DATA AS TO DISPOSITION OF GLANDS IN ALL FORMS UNDER INVESTIGATION SHOWING RESPECTIVE GLAND NUMBER BORNE ON LEAF BASE AND THE PETIOLE IN EACH SPECIES

Gland number	P. americana (wild); 877 leaves studied		P. americana mollis (Wolf); 400 leaves studied		P. Besseyi; 400 leaves studied		P. cerasus; 400 leaves studied		P. domestica (Shippers' pride); 400 leaves studied		P. pennsylvanica; 400 leaves studied		P. Simoni; 200 leaves studied		P. triflora (Burbank); 400 leaves studied	
	Glands on petiole	Glands on leaf	Glands on petiole	Glands on leaf	Glands on petiole	Glands on leaf	Glands on petiole	Glands on leaf	Glands on petiole	Glands on leaf	Glands on petiole	Glands on leaf	Glands on petiole	Glands on leaf	Glands on petiole	Glands on leaf
0	624	703	57	364	126	330	48	242	376	210	75	393	17	183	52	167
1	128	136	81	33	78	33	55	75	13	95	120	6	25	5	32	134
2	122	38	260	3	196	37	245	82	11	93	185	1	107	9	148	98
3	3	.....	2	.....	.....	.....	48	1	.....	2	16	.....	17	2	98	1
4	.....	.....	.....	.....	.....	.....	2	.....	.....	.....	4	.....	26	1	60	.....
5	.....	.....	.....	.....	.....	.....	2	.....	.....	.....	.....	.....	5	.....	9	.....
6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2	.....	1	.....
7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....

are rearranged in table III, to emphasize the modal class for gland number on the petiole and on the leaf base. It will be seen in all the combinations summarized therein that the mode for gland number on the petiole (with only three exceptions) is two, and that on the leaf base it is zero. In other words, the 2-0 condition shown in figs. 1 and 11 is typical in the plum. Thus it appears that the glands in the plum are too variable, both in position and number, for accurate statements concerning their disposition on

TABLE III

SUMMARY OF GLAND CONDITION IN ALL FORMS STUDIED, SHOWING MODAL CLASS OF GLANDS BORNE ON PETIOLE AND LEAF BASE.

Species or cross	Number of trees included in summary	Total leaf number	Glands borne on petiole			Glands borne on leaf		
			Number	Mode	Mean	Number	Mode	Mean
Abundance (*P. triflora*)	1	400	409	2	1.023	189	0	0.473
Burbank (*P. triflora*)	1	400	913	2	2.285	333	0	0.833
Wolf (*P. americana mollis*)	1	400	607	2	1.518	39	0	0.010
Abundance×Wolf crosses	38	15200	21956	2	1.440	9125	0	0.600
Burbank×Wolf crosses	22	8000	10406	2	1.300	5028	0	0.628
P. americana	3	877	356	0	0.406	212	0	0.242
P. Besseyi (Sand cherry)	1	400	407	2	1.018	107	0	0.268
P. Besseyi crosses	6	800	3359	2	1.866	788	0	0 438
P. Besseyi×*P. hortulana mineri* (Compass)	1	400	428	2	1.070	212	0	0.530
Compass crosses	8	1437	2019	2	1.405	514	0	0.357
P. cerasus	1	400	707	2	1.768	242	0	0.605
P. domestica (Shippers' pride)	1	400	35	0	0.088	287	0	0.718
P. domestica×*P. americana*	1	200	89	0	0.455	112	0	0.560
P. pennsylvanica	1	400	554	2	1.385	8	0	0.020
P. Simoni	1	200	438	2	2.019	33	0	0.165

any other basis than that of a statistical analysis. Other characteristics of glands, however, may be of distinctive value, and gland disposition may be sufficiently different and characteristic for a variety or species to be of taxonomic value. The outstanding feature of the data on the disposition of glands is that the mode for gland number on the petiole, with three exceptions, falls on two, and that the mode for the number borne on the leaf base in every instance is zero. This condition obtains notwithstanding the great diversity in the forms under investigation, and even in the three exceptions to a modal class of two on the petiole, the mode

was zero. This is significant in view of the fact that these three forms, *P. americana*, *P. domestica* (Shippers' pride), and *P. domestica*×*P. americana*, showed the greatest suppression of the glands of any of the forms included in this investigation. This condition will be given even greater emphasis in view of the connection found to exist between glands and the vascular system of the leaf.

Connection of petiolar glands with vascular system

With the status of the glands as to number and position shown by the statistical analysis in mind, it now remains to be seen whether or not there is a basic cause for the predominance of the 2-0 frequency.

The leaf trace in the plum has three bundles of conducting tissue at its departure from the vascular cylinder of the stem, which cause three gaps in the woody cylinder. The central bundle extends up the petiole, through the blade, and branches successively at the large lateral veins. The outer bundles give rise to strands which run along the upper side of the petiole, forming pronounced ridges on either side. These strands run directly to the petiolar glands, or to the large ones borne on the leaf base, and terminate there. The term petiolar gland, therefore, will be used in this connection to include both. The lateral strands are shown in fig. 30, which was drawn from a young leaf, and they are equally conspicuous whether the glands are borne on the leaf base or some distance down on the petiole. Where there is more than one gland on either side of the petiole, branches from the lateral strands connect with them.

On the other hand, the glands of the leaf serrations have a distinctly different vascular connection. Instead of being connected by branches with the lateral strands, glands in this position have their vascular connections with the central bundle through the lateral veins. It appears, therefore, that on the basis of differences in their vascular connections the petiolar glands and the glands borne on the serrations can be placed in two distinct classes. Other considerations also support this view. The petiolar glands are much larger than those on the serrations and may show differences in shape, such as the reniform or necked types,

which would readily differentiate them. Also under orchard conditions the glands on the petiole may be active much later in the season. That there is no relation between the glands in the two positions is further shown by the fact that in some species, as in *P. americana*, glands are typically absent on the serrations but present on the petiole or leaf base.

Glands which could not readily be classified as belonging to either the leaf serrations or the petiole were not numerous, consequently error from this cause has not entered to any appreciable extent into the statistical classification. There was also little difficulty in determining whether glands were borne on the petiole or leaf base, since in most cases there was no leaf tissue between the glands and the base of the blade. When leaf tissue was so present they were classed as being borne on the leaf base.

The question now arises as to whether the structure of glands on the petiole is similar to that on the leaf base. GREGORY (3) showed that glands borne on the petiole and leaf base in the peach were true glands, the upper part being composed of long rectangular cells rich in cytoplasm, and with large nuclei, while the central part is made up of parenchyma cells characteristic of glandular tissue, into which extend ramifications of conductive tissue. The structure of the glands in the plum borne on both the petiole and serrations has been examined on leaves just emerging from the bud, and on mature leaves with vigorous active glands, and is found to be similar to that reported by GREGORY in the peach.

The similarity in structure between the glands borne on the petiole and on the serrations led GREGORY to suggest that the former arose from the latter. Such an origin would imply that both are of the same rank, and that the tissue of the leaf blade is more or less indeterminate with that of the petiole, and would be in accord with the condition of the petiolar glands in some species of willow, notably *Salix lucida* (fig. 10), in which the glands are minute, numerous, and crowded together at the leaf base, suggesting a proliferation of leaf tissue along the petiole.

From these considerations it appears that there is justification for regarding the glands of the petiole, and the larger ones borne

on the leaf base, as of the same structure but of different rank from those borne on the serrations. This is in keeping with the evidence presented in the statistical analysis in which the number two figured so prominently, that is, two glands on the petiole and none on the leaf base, or one in either position, or less frequently, two on the leaf base. The points emphasized are of significance from the standpoint of phylogeny, and will be given greater emphasis from that standpoint.

Phylogeny of leaf as indicated by glands, vascular system, stipules, and abscission layers

There are structures other than the glands whch are significant from the standpoint of the ancestral type of leaf in *Prunus* and related genera. COOK (1) called attention to a joint in the leaves of Amygdalaceae "just above the insertion of the stipules," and states that the "basal section of the leaf below the joint" has a separate abscission from that of the leaf proper. This "basal section" is regarded as belonging to the leaf on the basis of the attachment of the stipules to it, although the stipules themselves absciss early, and hence "the persistence of the base of the leaf" has been overlooked. GOEBEL (2) characterizes stipules as "appendages arising at the insertion of a leaf, attached either wholly to the petiole or to the stem, or to both." SINNOTT and BAILEY (12) regard the stipules as arising through the stimulus of the growth of the lateral leaf trace, and although they are morphologically integral parts of the leaf, in some exstipulate families having trilacunar nodes, they are represented by mere swellings opposite the leaf trace. In some stipulate genera with opposite leaves, as *Viburnum*, the stipules arise midway between the insertion of the petioles, apparently directly from the stem. COOK also states that in Texas this basal section in the peach may remain alive for a year or two and then wither away; while in Maryland it lives through the winter and separates in the spring, leaving a fresh green scar.

In most of the plums studied in this investigation, this structure does not separate at all, but may be clearly observed (the petiole scar with its three bundle scars, and the two stipule scars at its

sides) on 3-year or even 4-year-old wood. Some vestige of it often remains a year or two longer, but usually after this time it begins to slough off.

It was not until some young trees of *Amygdalus Davidiana*, which were making an extremely rapid spring growth, were examined that the separation which COOK described was found. Ordinarily it requires some effort to remove the dry scalelike remnant left after the fall of the leaf, and usually there is more or less tearing of the bark; but in the case of *Amygdalus Davidiana* this structure separated easily and clearly when started with a knife point. In many cases after the rapid spring growth it cracked and separated without any outside stimulus, and later a large proportion fell as a result of further enlargement at the node. The condition is represented in fig. 5, which shows the separating scale, and fig. 31, which shows its structure in greater detail.

Examination of rapidly growing shoots of other *Prunus* species showed that a similar separation may take place, especially in some of the *P. Besseyi*×*P. triflora* hybrids, although in no case quite so sharply as in *Amygdalus*. At least a partial explanation of the fact that the separation of the scar scale occurs in some species of *Prunus* and not in others, can be made on the basis of the character of the swelling of the node below the leaf insertion. When it is straight in outline, it makes an acute angle with the line of the shoot and terminates in the leaf scar along a narrow ridge, as in *Amygdalus* (fig. 5). The rapid growth in spring of tissue underneath forces off the dead scale at the apex. If, however, the node is swollen, with a rounded profile, and the leaf scar is well buttressed below, as in the extreme type shown in fig. 4, the scale does not separate, and cannot be removed without tearing the bark. In either case, whether separation is immediate or delayed, it is only the shedding of dead tissue, just as bark is shed, and is done without the aid of a definite abscission zone (LOYD 9), and consequently is not true abscission.

A search for corroborative evidence as to the nature of this structure showed that in a number of genera it is not uncommon for clean cut separation of a scar scale to occur when rapid growth

begins. Some of the species which show the separation of the old leaf scar more clearly even than *Amygdalus* are *Shepherdia argentea*, *Cornus stolonifera*, *Tilia americana*, *Rhamnus cathartica*, and *Celtis occidentalis*, the first two of which are illustrated in figs. 16 and 17. All of these have simple leaves and lack petiolar glands, and *Cornus* and *Shepherdia* are exstipulate and have entire leaves, which, according to SINNOTT and BAILEY (**11, 12**), is an advanced state of node and leaf morphology. Hence we must regard the structure to which the stipules and petiole in *Prunus* are articulated, not as an additional foliar element, but as an outgrowth from the stem; and therefore abscission of this kind does not necessarily indicate an additional foliar element.

The manner of insertion and abscission of the stipules in the plum furnishes additional evidence that the structure described by COOK as a persistent leaf base is in reality a part of the stem, and that the leaves of Amygdalaceae cannot therefore be jointed.

In some Rosaceous genera, as *Potentilla* and *Rosa*, the stipules are adnate to the petiole, forming a somewhat sheathing base, and fall with the leaf. In others, as *Pyrus* and *Prunus*, the stipules are separate, or nearly separate, from the petiole, and absciss soon after the buds unfold. In *Prunus* the stipules usually drop long before the leaf, but occasionally they persist throughout the growing season, and even over winter, in vigorous, late growing branches in which cold weather has stopped further growth. The point of normal abscission is illustrated in fig. 32 (*P. hortulana mineri*). The stipules, like the petiole, separate at a definite abscission layer at their base (figs. 1, 2). As COOK pointed out, the joint of the latter lies above the stipules, that is, distally to them, although since the abscission lines of the petiole and the stipules form a sort of crescent with the points upward, a face view of the stem shows the stipule scars above that of the petiole (figs. 31, 32).

The typical leaves of many stipulate genera of the Rosaceae have stipules adnate to the petiole, forming a more or less sheathing base. This condition is also to be found in the bud scales and the scales transitional to leaves in *Prunus*. Morphologists regard bud scales as relatively primitive in structure, since they have not specialized to serve such varied functions as the leaves themselves.

Accordingly, the bud scales of *Prunus* may be taken as an index of the ancestral leaf type. A series of bud scales and scale leaves grading into true leaves is shown in figs. 18–29, which were taken from a young shoot of the Compass cherry (*P. Besseyi*×*P. hortulana mineri*).

It may clearly be seen that in the outer scales the stipules are represented by blunt lobes, as large as the central lobe which represents the leaf blade. The inner scales show progressive reduction in size of the lateral lobes and increase of the middle one, accompanied by differentiation into stipules and lamina. It will be noted, also, that there is progressive splitting of the stipules from the petiole. In the mature leaf of the plum this splitting has progressed to the base of the petiole, so that leaf and stipules have separate abscission, but from one originally continuous abscission layer. *Prunus avium*, as represented by the Dyehouse cherry, is at a somewhat intermediate stage. In the leaves near the base of a shoot, the stipules are clearly adherent to the petiole, while in the upper leaves they are separate, as in the plum. In all these transitional bud scales separation from the axis is clearly below the stipules, and there is no evidence of an abscission layer above them cutting off the middle lobe or leaf blade (fig. 2); hence the stipules in the plum must be regarded morphologically as integral with the leaf base, although the course of development separates them from the petiole in the mature leaf.

It remains to interpret the structure of the petiolar glands in relation to a more primitive type of leaf. Their organization and their occasional proliferation into leaflike outgrowths indicate that they are reduced structures. This change from a glandular to a foliar structure was noted by Cook (1), who described the occurrence of "small oblong or spatulate leafy organs on the upper part of the petiole, taking the place of the nectaries" in certain varieties of apricots. A similar transformation has been observed in *Crataegus*, as well as a number of species of *Prunus*, and in some of the apricot hybrids at the Minnesota Experiment Station it is of almost regular occurrence on vigorous shoots of young trees. Some of the variations in this transformation are illustrated in figs. 11–15. The view that the leaves of the Amygdalaceae are

jointed, led COOK to conclude that the ancestral leaf type of this group was compound, the nectaries representing "rudiments of divisions of compound leaves." It should be pointed out, however, that if the modern plum leaf represents the terminal leaflet of an originally compound leaf, the glands, representing reduced lateral leaflets, should be found below the joint by which the terminal leaflet is articulated to the rachis. It is obvious that being situated on the petiole above the joint, they cannot represent lateral leaflets of an ancestral pinnate form. Microscopic sections of the petiole at the leaf base do not show an abscission layer subtending the blade, and the presence of one is not indicated by the normal manner of shedding the leaves. It may be safely concluded, therefore, that the leaf blade of the plum is not articulated to the petiole, as in *Citrus*, *Berberis*, or *Trifolium*. Sometimes, however, if there has been late growth in the fall and the leaves are immature when the first frosts occur, the leaf axis may be broken anywhere from the middle of the blade to the base of the petiole, although most frequently at the juncture of the blade and petiole. In this case the twig enters the winter with a part or all of the petiole adhering at each node, which, however, usually breaks off at the point of normal abscission by spring.

Glands, however, may represent divisions of an alternate divided or pinnatifid leaf, such as *Fragaria*, *Potentilla*, and other genera of the Rosaceae possess. The frequency of the occurrence of two glands suggests a ternate leaf such as that of *Fragaria*. The presence of additional glands may be accounted for on the basis of branching of the lateral bundles, or by the common transition from ternate to quinquefoliate or pinnate leaves (LEWIS 7, 8). Some pinnate-leaved forms of *Potentilla* show reduction of the lower divisions to smaller structures than are the leafy outgrowths in the position of glands in the apricot. Furthermore, the lower divisions are frequently alternate instead of paired, as is also the case with the petiolar glands in *Prunus*.

It will be recalled that in the plum the two lateral bundles which connect with the glands terminate there, and do not contribute extensively to the vascular system of the blade. In ternate leaves which have three strands, the outer two provide the

vascular system of the lateral leaflets, and this is also frequently the case when there are numerous lateral leaflets, as in pinnate forms of *Potentilla*.

On foliar evidence the connection of the stone fruits, whether regarded as a tribe of the Rosaceae or as a separate family, with the Potentilleae is quite direct. The nodal anatomy is the same (SINNOTT and BAILEY 12) and the steps in leaf evolution appear to be: (1) reduction of the lateral leaflets of a ternate or pinnate-divided leaf to petiolar glands; and (2) splitting of persistent adnate stipules from the petiole. In this series *Prunus avium*, with an extensive development of glands, both in number and size, or the apricot with a frequent reversion to leafy structures in place of glands, would stand below *Prunus americana* with its almost glandless petioles. GREGORY (3) regarded the globose gland as more primitive than the reniform, since when normally glandless leaves produced glands, they were always of a globose type. If, however, the trend is toward reduction of glands, the glandless petiole would be the highest type, and the globose condition transitional between it and the reniform.

It has been customary to trace the connection of the drupes with the more primitive Rosaceae through *Spiraea* (RYDBERG 10). Considered on the basis of floral evidence alone, this seems a logical sequence, *Spiraea* being intermediate in form of receptacle and number of carpels between *Potentilla* and *Prunus*. SINNOTT and BAILEY (12) have shown, however, that *Spiraea* is exstipulate and possesses a unilacunar node, while all species of *Prunus* have stipules and a trilacunar node. It would appear that forms with stipules could not be derived from forms which lack them. The relationship of the stone fruits to the true Rosaceae is probably more direct, and on the basis of anatomy there is less reason for separating them as a distinct family than for considering them a well defined and specialized tribe.

Summary

Examination of over 30,000 leaves belonging to 15 species and interspecific hybrids of the plum shows that two glands typically occur on the petiole, or less frequently on the leaf base.

On the basis of vascular connections the glands on the petiole or leaf base are of a different order of structure from those on the leaf serrations.

The stipules in the plum are morphologically integral with the leaf base, and separate from the stem by a common abscission layer. A portion of the node bearing the leaf and stipule scars is subsequently shed in some species of *Prunus* as in other woody genera; but the portion thus shed is not an additional foliar element.

On the basis of nodal anatomy and the presence of reduced structures, the ancestral type of leaf in the plum is considered to be a ternate lobed or divided simple leaf, the petiole glands representing the suppressed lateral members. In floral structure and nodal anatomy *Prunus* and related genera form a logical series with the Potentilleae, and should be considered as a specialized tribe of the Rosaceae.

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EXPLANATION OF PLATES XX, XXI

PLATE XX

FIG. 1.—Section through node showing abscission zone by means of which the leaf is shed; *a*, only abscission zone present.

FIG. 2.—Showing continuous abscission zone (*a*) for stipule and petiole at early stage of growth.

FIG. 3.—Dark line at base of stipule showing point of abscission; note that it is distal to attachment of petiole.

FIG. 4.—Instance of extreme swelling in node below point of abscission of leaf; in such instances scar scale is not shed.

FIG. 5.—Enlarged view of shedding of scar-scale (*a*) in *Amygdalus Davidiana* as result of early spring growth; shedding of this type occurs only when contour of node is relatively flat.

FIGS. 6–9.—Variation in gland position and number; types illustrated are 2-0 (2 on petiole and 0 on leaf base), 1-0, 1-1, and 0-1.

PLATE XXI

FIG. 10.—Leaf of *Salix lucida* showing glands similar to those of leaf serrations crowded at base.

FIGS. 11–15.—Types of proliferation of petiolar glands into leafy outgrowths.

FIG. 16.—Node of *Shepherdia argentea* showing shedding of scar scale similar to *Amygdalus*.

FIG. 17.—A node of *Cornus stolonifera* showing same as 16.

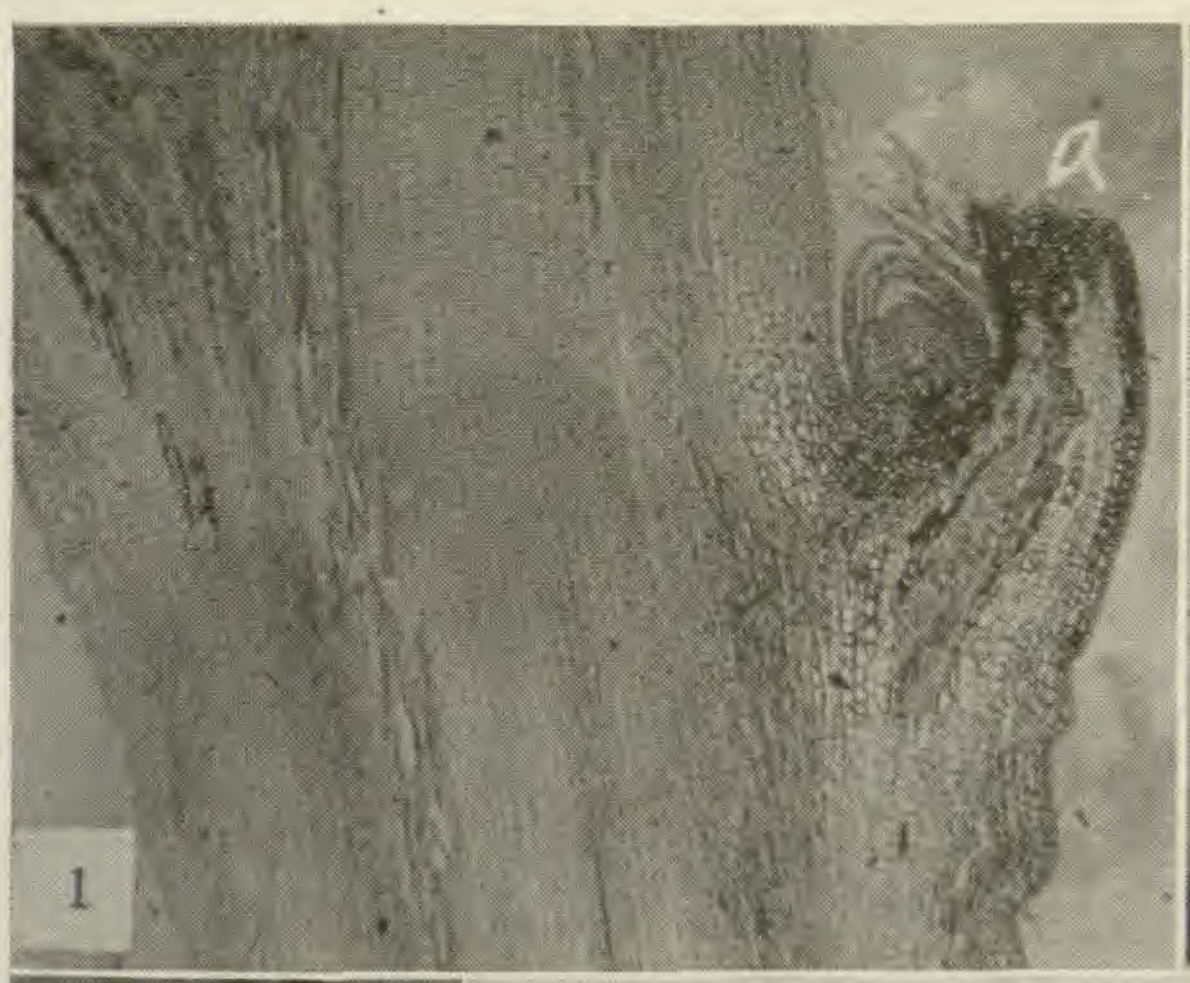
FIGS. 18–28.—Bud scales and basal leaves from young shoot of Dyehouse cherry, presenting series in separation of stipules from leaf blade.

FIG. 29.—Enlarged basal leaf illustrating venation of stipules and leaf blade; note that veins of stipules arise as branches of lateral strands.

FIG. 30.—Petiole showing prominence of lateral bundles in young leaf.

FIG. 31.—Shedding of dead scar scale in *Amygdalus Davidiana* from which stipules and petiole abscissed previous season.

FIG. 32.—Node of young plum stem showing relation of line of abscission in stipule and petiole.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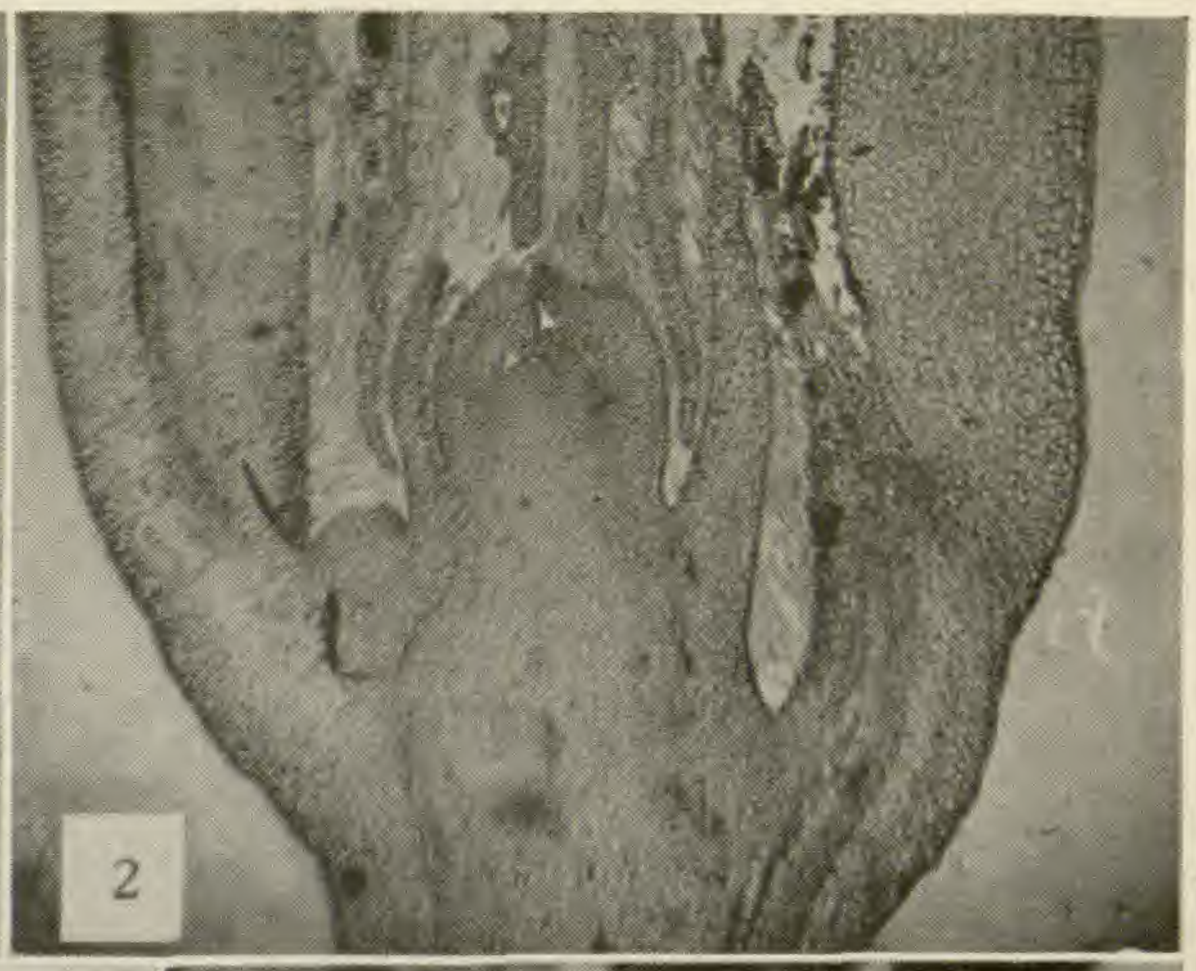
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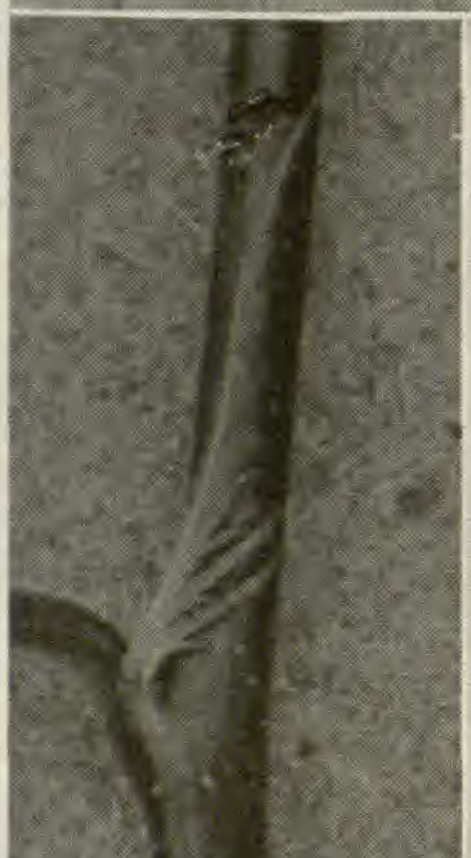
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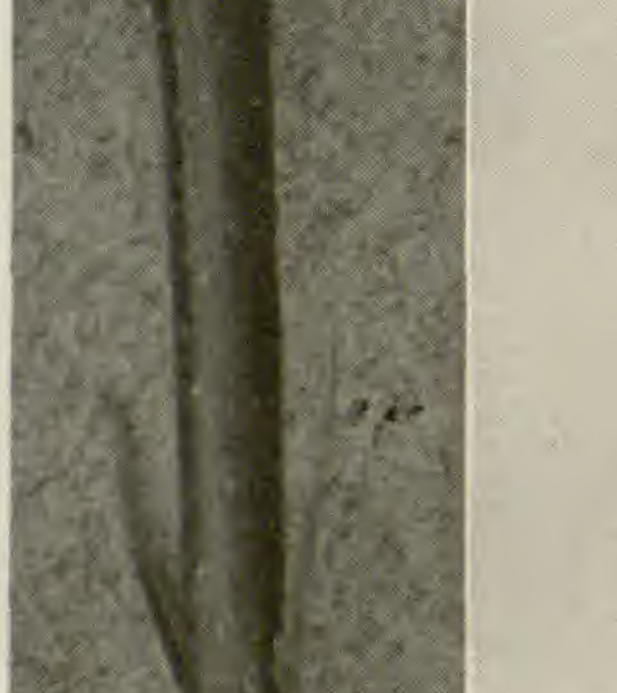
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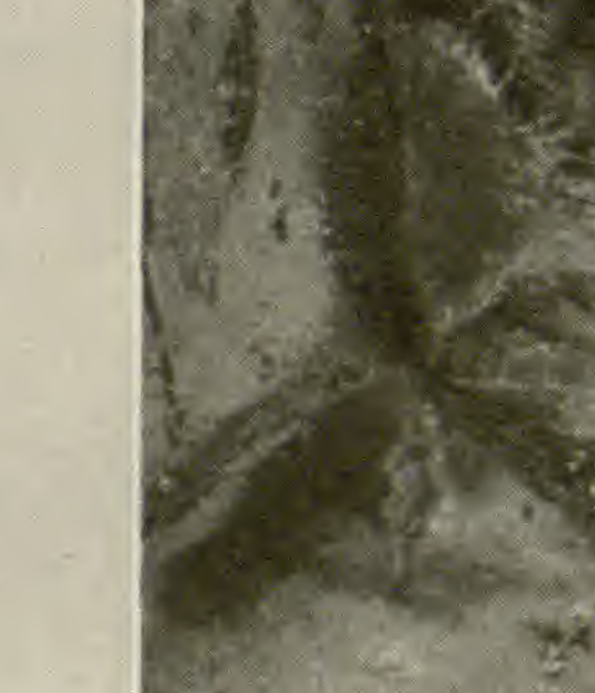
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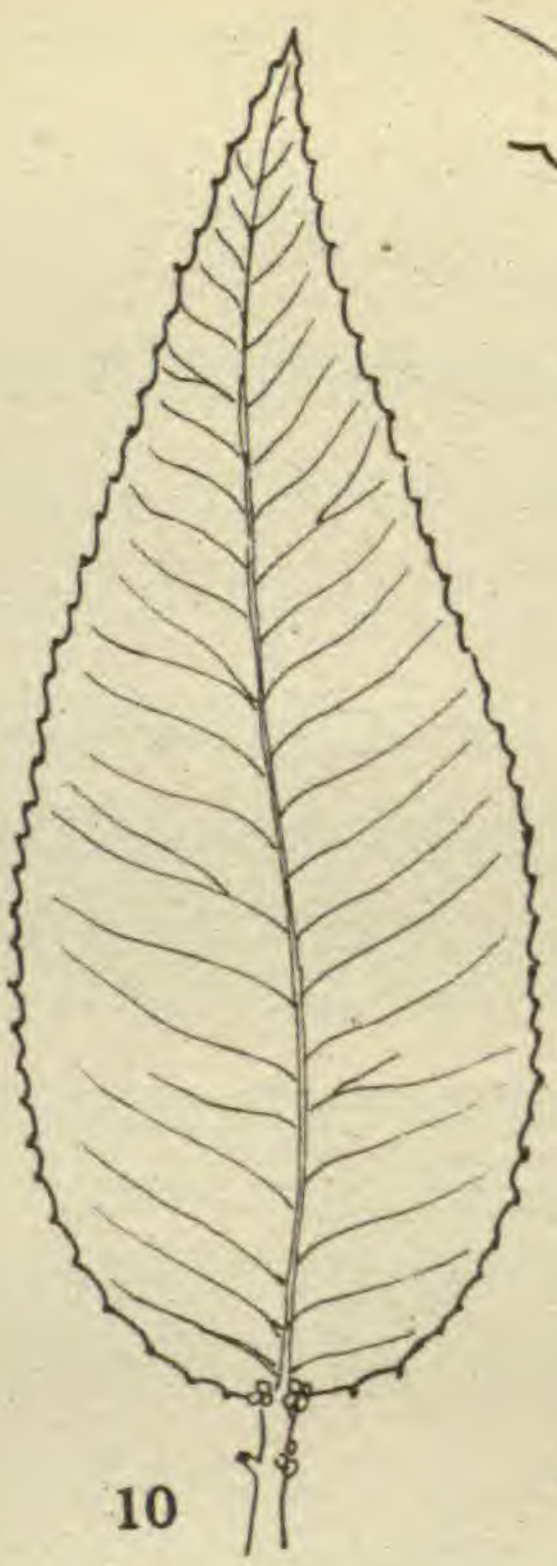
DORSEY & WEISS on GLANDS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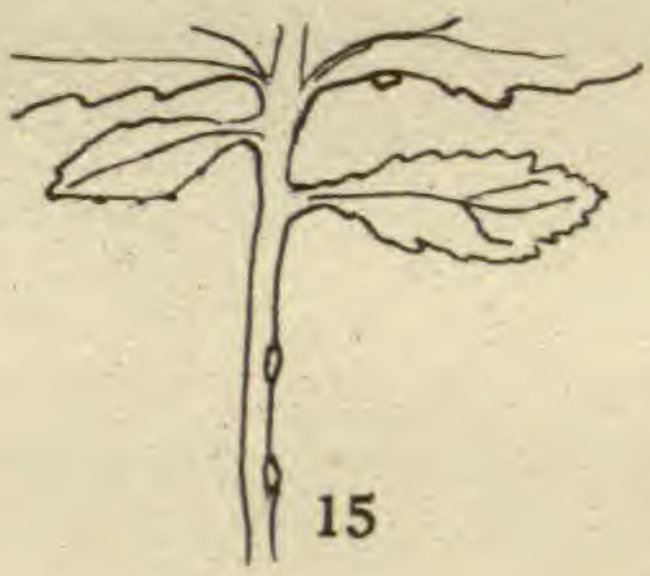
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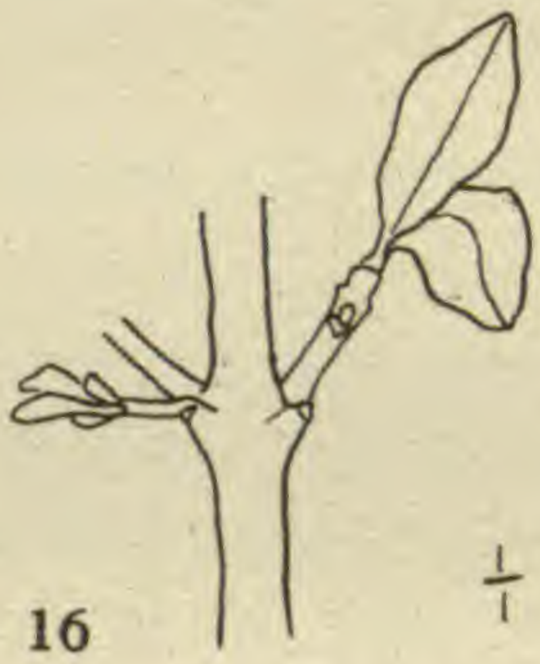
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DORSEY & WEISS on GLANDS

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.

UNIVERSITY OF CHICAGO

BULBILS OF LYCOPODIUM LUCIDULUM

R. Wilson Smith

(WITH TWENTY-ONE FIGURES)

It is well known that certain species of *Lycopodium* belonging to that subdivision of the genus which is characterized by the non-strobilar arrangement and slight differentiation of the sporophylls produce organs of vegetative reproduction known as bulbils or gemmae. These organs are of a peculiar type, so unlike anything else in the plant kingdom that the question of their morphological nature is a very puzzling one. Various opinions have been held. They have been interpreted as equivalent to sporangia, to lateral branches in the axils of leaves, to lateral branches without supporting leaves, to reduced branches resulting from dichotomy of the stem apex and gradually displaced, and to the bulblets of certain ferns and monocotyledons.

We owe the first accurate account of the development of the bulbils to Hegelmaier (4). He corrected the view previously held that they originate in the axils of preexisting leaves, and showed that they take the place of leaves in the phyllotactic spiral. He could find no means of distinguishing very young leaves and bulbils in longitudinal sections; and a view of the apex of the plant showed a difference only when the larger size indicated that a bulbil was beginning to develop.

Strasburger (10) gave a short description of the bulbils, differing from Hegelmaier's in some respects. In the following year, after further investigation (11), he confirmed the interpretation of Hegelmaier in every detail but one; he thought leaves and bulbils differ somewhat in their mode of development. By special manipulation of the stem tips, he selected for study those tips on which young bulbils were present. In these young bulbils, when viewed in longitudinal section, he thought he could detect a middle apical group of two cells in the dermatogen. This account does not contradict that of Hegelmaier, for Strasburger really found no difference between leaves and bulbils until the latter

were distinguishable by their greater size. My observations on this feature agree with HEGELMAIER'S. I have not been able, in longitudinal sections, to distinguish a young bulbil from a young leaf until it is recognizable by the beginning of an apical meristem at its tip. STRASBURGER discussed the homology of the bulbil with considerable care, and after considering the possible explanations concluded that all the facts of development and histology are best combined in the supposition that the bulbil represents the survival of an original dichotomy ("eine einst dichotomischen Ursprung").

Much the same interpretation is given by CAMPBELL (1), who says the bulbils "are formed apparently in the axils of somewhat modified leaves. . . . . The axillary origin of the bulbils is only apparent; they are really, so far as can be determined, similar in origin to ordinary branches and formed without any relation to the leaves."

GOEBEL (3) considered the bulbils from the point of view of their adaptations rather than their homology. Although disclaiming any attempt to solve the latter, he brought forward some pertinent objections to current morphological explanations, and concluded that in their most important features the bulbils of *Lycopodium* are not unlike the bulblets of certain species of *Allium* and *Lilium*.

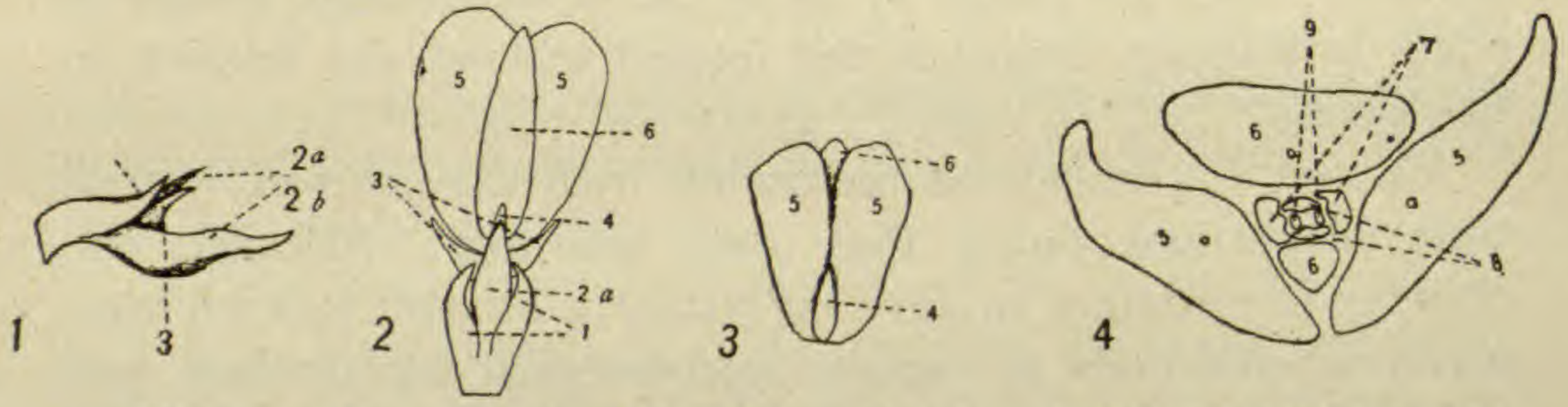
The present investigation is an attempt to help solve the problem of the bulbils by means of modern technique and serial sections which were not available to the earlier investigators. Only *L. lucidulum* has been studied, suitable material of other species not being obtainable. STRASBURGER, however, found that there is a great similarity in the bulbils of the six species which he compared, and it is probable therefore that the following account with only minor changes will hold good for all.

Description of bulbils

Two parts must be distinguished in the bulbil: the base which remains attached to the stem of the plant for many years and does not store up nutriment; and the distal part, or bulbil proper, which is heavily laden with starch grains, and which, when mature,

separates from the base and readily gives rise to a new plant. Between the two parts is a narrow neck which breaks easily when the bulbil is ripe. The young bulbil has a small apical meristem at its tip, and as it grows produces decussate pairs of protuberances, which in their relation to the bulbil axis resemble foliar organs, but in maturity differ more or less from typical leaves. The base has three pairs of these and the bulbil proper about six.

From the bulbil primordium there arise, as described by HEGELMAIER from external appearances, first a pair of small leaves, laterally situated, but soon displaced toward the stem apex; then from the growing point between these a median pair, and soon after them another lateral pair, not displaced. The median pair, especially its abaxial member, is much the largest. This larger



FIGS. 1–4.—Fig. 1, side view of bulbil base; ×3; fig. 2, bulbil and base from upper side; ×3; fig. 3, bulbil proper from lower side; ×2.5; leaves numbered in order of appearance; *2b*, "supporting" leaf; fig. 4, cross-section of tip of bulbil; leaves numbered in order of appearance; ×10.

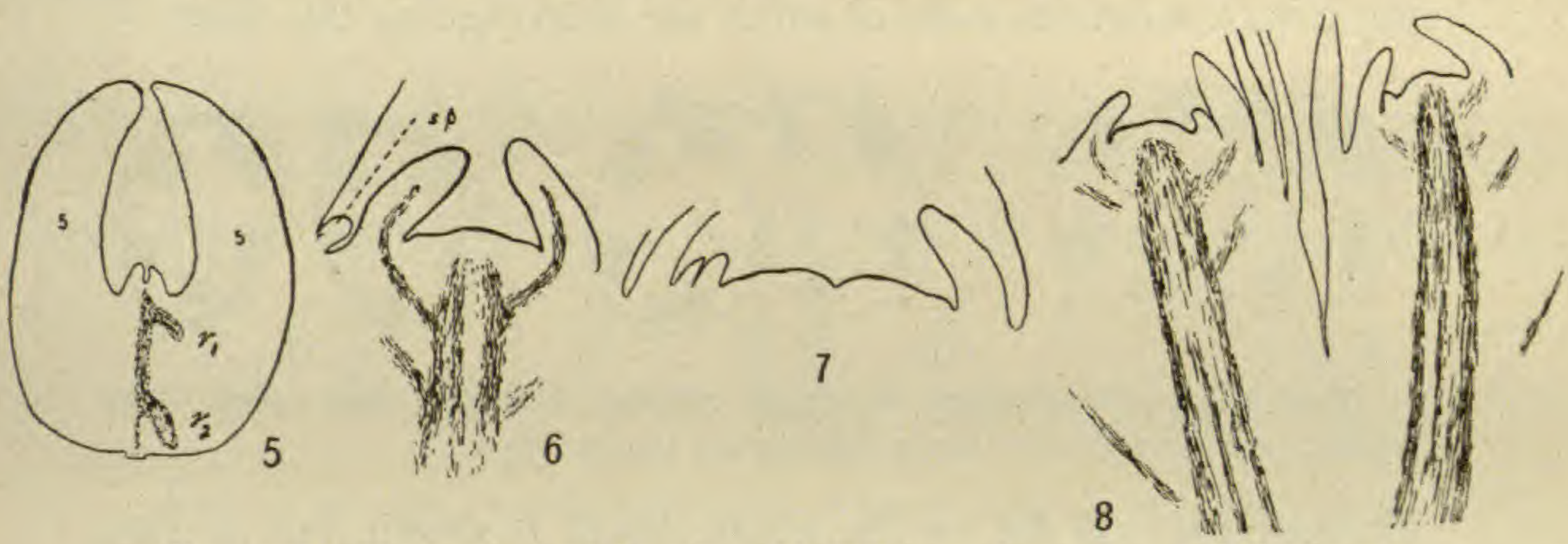
outer leaf becomes the so-called "supporting" or "cover" leaf of the bulbil (figs. 1, 2). HEGELMAIER was unable to decide whether the further axis of the bulbil is the direct continuation of the primordium, and the supporting leaf therefore truly lateral, or whether the supporting leaf is the direct continuation of the primordium, and the bulbil proper therefore an outgrowth and truly lateral.

The first leaves of the bulbil proper are dorsal and ventral; the second pair, which are lateral in origin, gradually turn their upper edges outward and assume a dorsiventral relation; the third pair are median, long, and narrow (figs. 2, 3). These three pairs of leaves are fleshy, and the leaves of the second pair are also much modified in form. The remaining leaves of the bulbil

are small and closely arranged about the growing point (fig. 4). The axis of the bulbil proper develops endogenously two small rootlets, which remain imbedded in the fleshy leaves until the germination of the bulbil, when they push out into the soil.

Anatomy of stem apex and bulbils

L. lucidulum has only one mode of branching, namely, by dichotomy of the apex. The growing point of the stem tip is broad and flat, or slightly raised in the center, and is surmounted and protected by the more rapidly lengthening leaves. In *Lycopodium* there seems to be a relation between the form of the growing point and the rate of growth. Slow growers, such as *L. lucidulum* and *L. Selago*, have the apex as described (fig. 6); while rapid



FIGS. 5–8.—Fig. 5, tangential section of bulbil: r_1 and r_2, roots; *s*, fleshy leaves; ×5; fig. 6, apex of stem: *sp*, young sporangium; ×38; fig. 7, beginning of dichotomy of stem in longitudinal section; ×38; fig. 8, more advanced stage of same; ×23.

growers, such as *L. clavatum*, *L. annotinum*, and *L. alopecuroides*, have the apex conoid in form and considerably in advance of the youngest leaves. Before the apex branches it becomes wider (there is considerable variation in the width of the growing point without any apparent relation to branching). The initial cells of the middle region now cease to grow and multiply, while those on either side continue their activity (fig. 7). Soon the two growing points, separated by a cleft, begin putting forth leaves on all sides (fig. 8). In longitudinal section it can be seen that the central cylinder has divided into equal strands, and the two apices are similar in all respects.

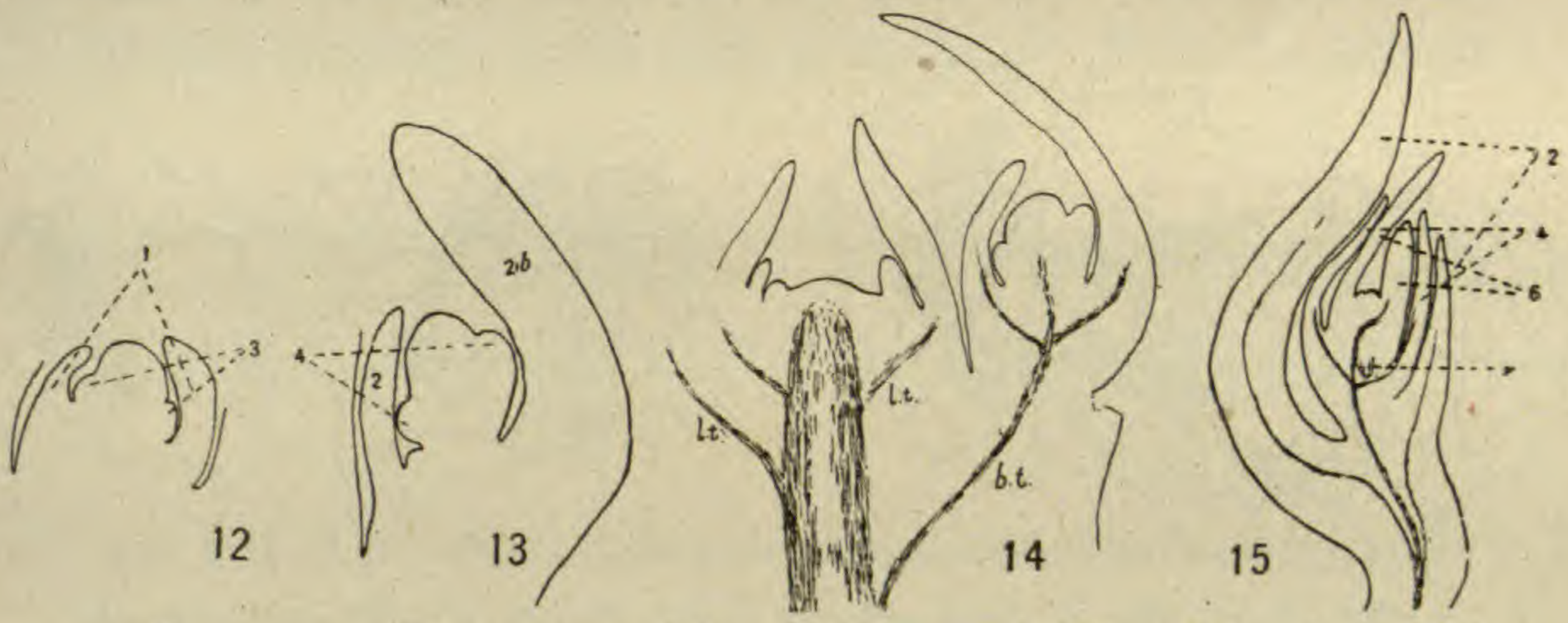
In cross-sections the equality of the two branches is just as apparent. Fig. 9 shows a section of the central cylinder below a dichotomy at a distance of about half a year's growth from the two tips. The central part of the cylinder is filled with immature tissue, which can be distinguished approximately into xylem and phloem areas. The shaded areas represent fully differentiated xylem made up on the outer side of protoxylem tracheids, and on the inner side of larger metaxylem elements. As differentiation proceeds in this species, the metaxylem portions meet in the middle of the cylinder and become more or less continuous, and the phloem occupies the indentations or bays between the xylem strands. Surrounding the cylinder there is a clearly marked endodermal sheath in the form of an irregular layer of cells, only the inner contiguous walls of which are thickened at this stage of

FIGS. 9–11.—Cross-sections of central cylinder of forking stem, xylem areas shaded; small cross-hatched areas indicate leaf traces; ×52.

development. In fig. 11 the whole sheath is shown, but in figs. 9 and 10 only the thickened cutinized walls are to be seen. As the dichotomy proceeds the xylem strands change position but little, the sheath from opposite sides pushing inward until the division into two similar cylinders is complete. In this mode of branching no branch gaps of any kind are formed in the sheath or xylem or phloem. The length of stem between figs. 9 and 10 was 0.65 mm.; and between figs. 10 and 11 it was 0.5 mm. It will be noted that there are eight xylem strands in fig. 9, and that in each of the two branches there are five. The new strands are not formed by a division of old ones, but arise *de novo* out of meristematic tissue. The beginnings of these strands may be seen in fig. 10.

As shown by JONES (**5**), the protoxylem strands in *Lycopodium* are constant neither in number, shape, nor position. New strands

may arise either by branching of preexisting strands, or from the meristem. I have also found that a strand when traced upward may gradually diminish in size and ultimately disappear. In the stem of which figs. 9, 10, and 11 are sections, the leaves were 9-ranked, and this ranking continues in both the branches, notwithstanding the reduction of protoxylem strands from nine to five. As shown many years ago by CRAMER (2) and HEGELMAIER (4), there is no relation between the number of orthostichies and the number of xylem strands. The leaf traces of *Lycopodium*, so far as they have been investigated, are mesarch in structure (7, 8). They are so in *L. lucidulum*. The trace separates very gradually

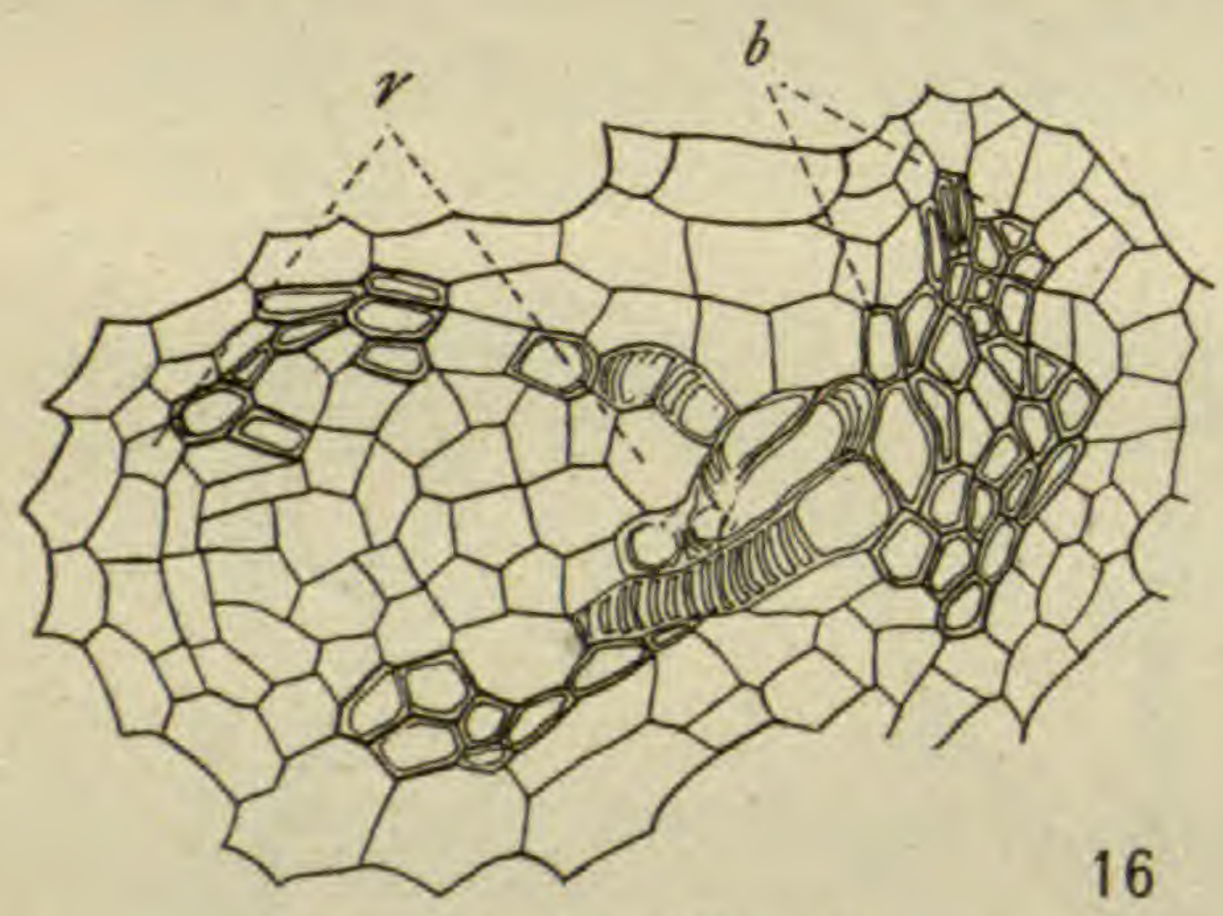


FIGS. 12–15.—Fig. 12, tangential section of young bulbil: *1*, first pair, *3*, third pair of lateral leaves; fig. 13, radial section of bulbil rather older than fig. 12; *2*, first pair of median leaves; *4*, first leaves of bulbil proper; ×38; fig. 14, longitudinal section of stem tip and young bulbil: *2b*, "supporting" leaf; *lt*, leaf trace; *bt*, bulbil trace; ×23; fig. 15, longitudinal section of older bulbil, leaves numbered in order of appearance; *r*, root tip; ×10.

from the protoxylem of the central cylinder, traverses the pericycle for a short distance, and then pierces the endodermis, after first pushing it out into the form of a little pocket (figs. 10, 11). The separation of the leaf trace causes no gap or disturbance in the central cylinder. Immediately above and below the point of exit the sheath closes. The leaf trace at first consists only of spiral tracheids, but later is surrounded by deeply staining cells, presumably phloem.

The vascular strand of the bulbil does not at all resemble that of a branch. It is a single strand, similar in every way to a leaf

trace in its relation to the central cylinder and its passage through the cortex (fig 14). Not until it reaches the outer part of the cortex or the decurrent base of the bulbil does it exhibit any essential deviation from a leaf trace. Here it widens into a narrow tangential band of cells, and presently gives off laterally two small traces to the first leaves of the bulbil (fig. 12). The middle part continues and presently widens into a radial band whose outer portions become the traces of the second pair of leaves (figs. 13, 15), and so on until four pairs of traces have separated from it. These facts make it clear that the "supporting" leaf is undoubtedly a lateral organ. In the bulbil proper in connection with the appearance of



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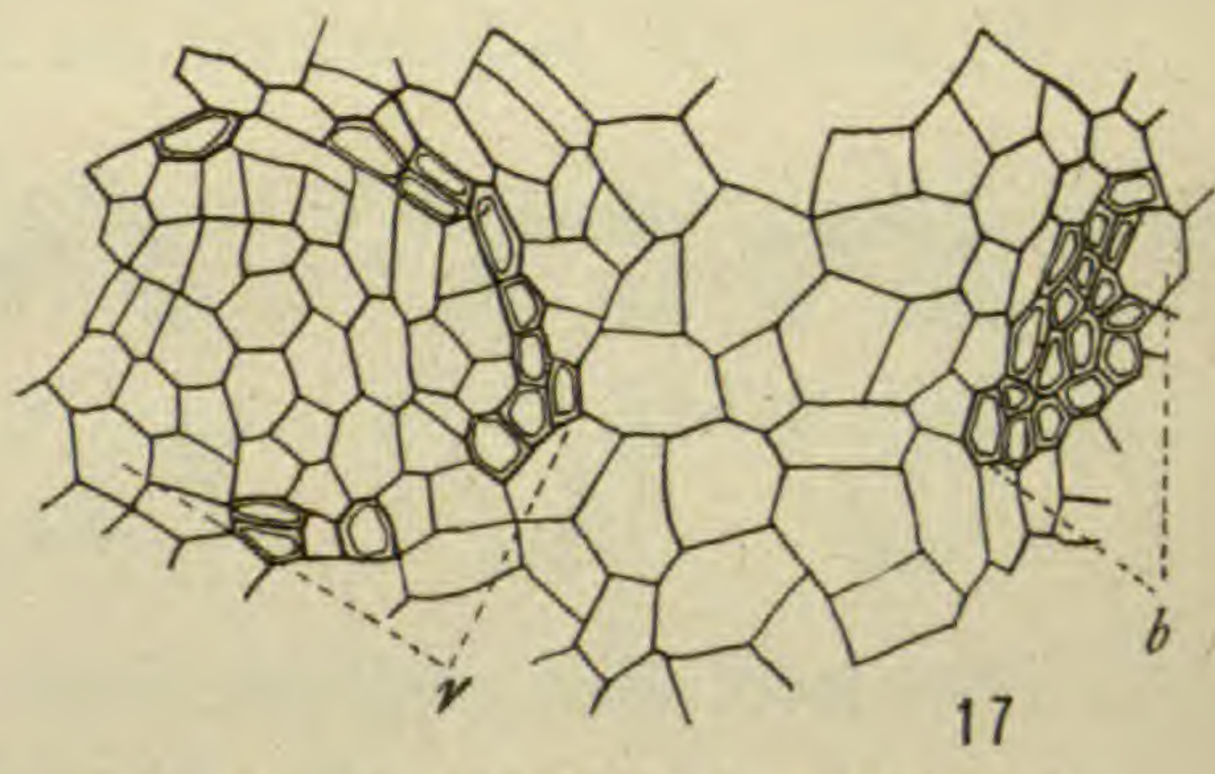

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FIGS. 16, 17.—Fig. 16, cross-section of vascular bundle of bulbil where first root is given off; ×320; fig. 17, cross-section of bundles of bulbil and root below origin of root: *b*, bulbil bundle; *r*, root bundle; ×320.

the first root a much greater change occurs. In a young bulbil, even before the vascular tissues are differentiated, the tip of this root is outlined as a mass of deeply staining meristematic cells. A second and smaller root develops nearer the tip of the bulbil (fig. 5). Both of these roots connect with the vascular strand of the bulbil on its adaxial side and, turning downward and laterally, remain imbedded in the parenchyma and separated from it by slime and crowded tissue.

Fig. 16 shows the junction of the root cylinder and the axis of the bulbil, and fig. 17 is a section below this. In both of these it can be seen that the bulbil axis is still essentially a leaf trace, but above the junction of root and axis all resemblance to a leaf trace is lost. The arrangement of the tracheids is somewhat like

that of a root, but much more irregular. Sometimes it is distinctly diarch (fig. 18), more commonly it is horseshoe-shaped, and sometimes almost a complete ring, often with a few tracheids in the center (fig. 19). Surrounding the whole xylem area there are one or two irregular layers of cells, probably a pericycle, and outside these an indistinct sheath (this feature is shown only in fig. 16). This irregular arrangement continues until the fifth and sixth pairs of leaf traces are given off and also the second root. Above that the tissues are still meristematic in the ripe bulbil. The bulbil, like the leaves, is well supplied with stomata, and photosynthesis proceeds actively. The cause of the accumulation of starch in the bulbil proper and not in the base seems to lie in the absence of phloem in the neck of the bulbil. Here the cells are

FIGS. 18, 19.—Cross-sections of bundle above origin of first root; ×320

entirely wanting which elsewhere in the bulbil surround the xylem strand, and which I have regarded as phloem, although the sieve plates have not been identified. The detachment of the bulbil proper is brought about by a disorganization, apparently a gelatinization, of the walls of the xylem cells in the neck.

When a ripe bulbil is kept on damp soil it soon germinates. Both roots penetrate the soil and branch repeatedly, and from the tip of the bulbil a slender stem is put forth. On this stem the new leaves, although scattered, are from the first arranged spirally. JONES (5) traced the development of the vascular cylinder in the young plants growing from bulbils of *L. Selago* and *L. serratum*, and concluded that the original simple bundle becomes successively diarch, triarch, etc., by division of the xylem. My observations on *L. lucidulum* do not agree with this conclusion. I find, in agreement with JONES, that the young stem at its attachment with

the bulbil is diarch, having two masses of xylem with one phloem mass between, but followed up for nearly an inch, it never becomes triarch. The xylem becomes horseshoe- or ring-shaped, much as in figs. 17 and 19, changing constantly, and there is no hint as to how the multiprotoxylic condition of the adult stem arises. The irregularity and continual change in the xylem of these young stems are comparable to the conditions described by Miss WIGGLESWORTH (12) in young *Lycopodium* sporophytes growing from gametophytes.

HEGELMAIER'S statement that the bulbils replace leaves in the phyllotaxy is confirmed by an examination of the relation of bulbil and leaf traces. On following these back in cross-sections to their junctions with the xylem strands of the central cylinder, the bulbil traces are found in close succession. In many cases they represent successive leaves; in other cases a leaf trace stands between two bulbil traces; but in every case examined the bulbil traces all fell within a single leaf spiral.

Conclusions

1. The bulbil is not the homologue of a branch, since it has a simple vascular strand with mesarch concentric arrangement; whereas branches have a vascular system with complex exarch radial arrangement. Furthermore, it is not a reduced dichotomy or the equivalent of the bulblets of *Lilium* or *Allium*.

2. It is not the homologue of a sporangium, for the reason, among others, that it receives a prominent vascular strand, a feature which is lacking in the sporangia of all Lycopodiales.

3. It is a transformed leaf, retaining the position, dorsiventrality, and in great measure the vascular strand of a leaf. It may perhaps be homologized with the bulblet of a fern produced so early in the history of the leaf bearing it that the latter exhibits the leaf character only while inside the cortex of the stem.

Further observations on the bulbils and habits of L. lucidulum

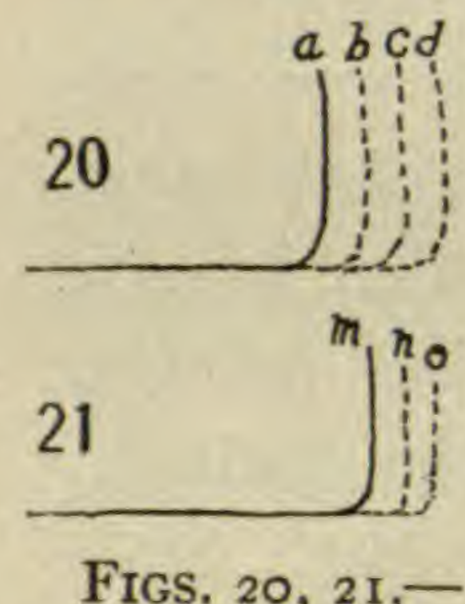
In the literature of the subject, I do not find any recognition of the periodicity of the bulbils. In *L. lucidulum* they are quite as responsive to season as the sporangia. The latter begin to form

in autumn in preparation for the next year, and continue to form when growth is renewed in the spring. Then, beginning some time in June, for more than two months the apex develops a succession of sterile leaves, in the midst of which two to four bulbils may make their appearance, always near each other and on one side of the stem, on that side of a branch which is farthest from the other branch. The primordia of the bulbils increase rapidly in size, pointing vertically upward at first like the young leaves, and then gradually becoming horizontal. They reach maturity in September. Since the bulbils are formed but once a year and their bases are persistent, it follows that they furnish a means of measuring the annual increment of growth in length. There are other but less accurate means of estimating a year's growth, by the old sporangia whose walls do not completely disappear for several years, and also by a difference in the length of sporophylls and sterile leaves. The annual growth in plants bearing bulbils can be determined quite easily for the last ten or twelve years, and in some cases even for twenty years. It is surprisingly small, averaging about one inch.

The possibility of determining the age of any given part of the stem leads to some interesting observations. For instance, it can be shown that the metaxylem matures very slowly, not being fully differentiated until well into the second year. The cortical zones, characteristic of most *Lycopodium* stems, are not differentiated until the third year. It is also possible to calculate the frequency of dichotomy of the stems. The commonest interval between successive dichotomies is four years, but intervals of two, three, five, and six years also occur frequently. I have never found a stem branching in two consecutive years.

Like several other species, *L. lucidulum* has the old stem horizontal and rooted, and the young and greener stem upright. By means of the bulbil bases it is clearly shown that the upright part is six or seven years old. Observations from year to year show that the plants, although adding annually one inch to their length, maintain the same average height. Each year a part of the erect stem, equaling a year's growth, becomes curved and finally horizontal. Fig. 20 represents the situation in four

successive years. Decapitated plants are not killed, but, having no means of renewing the apical meristem, they cease to grow or divide. Fig. 21 represents such a decapitated stem in three successive years, the upright portion shortened each year by the equivalent of a year's growth. Whether such a stem will finally become altogether horizontal, I do not yet know, nor have I yet been able to discover the cause of the regular change in direction from upright to horizontal. Observations in the field seem to show that root contraction is not the determining cause (stem tips decapitated in 1915 were found to be dead in the summer of 1919).



FIGS. 20, 21.—Fig. 20, diagram representing position of plant four successive years; fig. 21, diagram representing position of decapitated plant three successive years.

Some interesting data can also be gathered in regard to the behavior of the roots. These, as in many other species of *Lycopodium* whose tips are erect or sloping (**6, 7, 9**), do not pass straight out through the cortex, but turn downward. In *L. Selago* these roots "arising at the apex pass obliquely, then directly down through the middle cortex and only appear at the outside beneath the soil" (**6**). The same description is true of *L. lucidulum*, except that the roots do not originate at the apex, but begin at some distance behind the apex; how far behind is not clear. They rarely form within the first year's growth. Apparently they increase in number in the older parts of the erect stem; but as small roots might easily be overlooked in freehand sections, I am not quite certain that new roots may form in parts of the stem as old as five or six years. These roots, in the upright part of the stem, remain small, usually less than 6 mm. in length. As they do not emerge to the outside until in a part of the stem at least six or seven years old, it is evident that they must be in a dormant or slowly growing condition within the cortex for as many as two to five years. The downward growth in the cortex is not very great, the distance between the junction with the central cylinder and the point of emergence to the outside varying from 6 to 15 mm. Reaching the soil, they grow rapidly, quickly reaching a length of several inches.

Summary

1. The origin and vascular bundles of branches, leaves, and bulbils are described, and the conclusion is reached that the bulbil is comparable to a leaf rather than to a branch.

2. The accumulation of starch in the bulbil proper is ascribed to the absence of phloem in the narrow neck joining it to the base, and the detachment of the ripe bulbil to the disorganization of the xylem walls in this region.

3. The rate of growth is estimated from the persistent bases of the bulbils, and observations are made on the habits of the plant.

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TENDRILS OF SMILAX[1]

AGNES ARBER

(WITH PLATE XXII)

The nature of the tendrils of *Smilax* has excited perhaps more controversy than almost any other problem arising out of the normal vegetative morphology of flowering plants. It is unnecessary to deal here in detail with the voluminous literature which has grown up about the subject, since this task has been excellently performed by DOMIN (4), who himself holds the opinion that the tendrils are trichome structures, an opinion which does not seem to the writer to be well founded. It will suffice to group together the various alternative views which he has enumerated, and to attempt to test them, from the standpoint of the phyllode theory (1), by a study of a typical species, *Smilax herbacea* L. (fig. 1). The views which different authors have promulgated may be considered as follows, under five heads:

1. That the *Smilax* tendrils are metamorphosed stipules, or outgrowths of the apical region of the leaf-sheath.

Both these possibilities seem to be excluded by the fact that the tendrils arise from the petiole, immediately above the sheath, it is true, but entirely disconnected from it both externally and as regards their vascular system.

2. That the *Smilax* tendrils represent a bipartite ligule, each tendril being a demi-ligule.

This alternative is ruled out because, as GLÜCK (5) and DOMIN (4) have shown, there is occasionally a definite ligule in addition to the tendrils. It is also discounted for the same reasons that make the first view untenable.

3. That the *Smilax* tendrils are either metamorphosed leaflets, segments, or lobes of a compound leaf, or lateral free nerves which become detached before the others.

[1] This paper represents part of the work carried out during the tenure of a Keddey Fletcher-Warr Studentship of the University of London, and with the aid of a grant from the Dixon Fund of the University of London. The writer wishes to acknowledge her indebtedness for material to Miss G. Lister, Professor Seward, and the Curator of the Cambridge Botanic Garden.

If any of these related alternatives held good, we should expect to find the vascular structure of the tendril showing some affinity with the vascular structure of the midrib or lateral veins of the "lamina" of the *Smilax* leaf. The midrib and lateral veins of the blade, however, are each characterized by an arc of bundles (fig. 4), while the tendrils have an irregular closed ring (fig. 3 *B*).

4. That the *Smilax* tendrils are metamorphosed trichomes or emergences.

The high development of the vascular system of the tendrils seems to make it impossible to accept this theory. The emergences on the stem of *Smilax* itself, which on this view must be homologous with the tendrils, are non-vascular, as are the paired glands at the base of the leaf of *Tamus communis* L., whose position at first glance suggests a comparison with the tendrils of *Smilax*.

5. That the tendrils of *Smilax* represent "un double prolongement latéral des éléments cellulo-vasculaires du pétiole."

This view, which was suggested by CLOS (2) more than half a century ago, and has been more recently supported by GLÜCK (5), seems to contain the germ of the true explanation, although, in the form in which CLOS enunciates it, it is essentially descriptive rather than morphological. The writer wishes to propose a related but more comprehensive view, which interprets each of the tendrils of *Smilax* as equivalent in morphological value to the petiole, and as having originated through a dédoublement or chorisis of that organ.

An analogy may perhaps be suggested with the stamen phalanges of *Hypericum Elodes* Huds., which sometimes consist of three members, and which very probably have arisen by secondary chorisis of an ancestral single stamen. A closer analogy is indicated by QUEVA'S (7) comparison between the anatomy and insertion of the tendrils of *Smilax* and the stalks of the leaflets of certain Dioscoreaceae with compound leaves. It seems by no means impossible that, in this family, the compound character of the leaf may also be due to chorisis of the petiole giving rise to three equivalent organs.[1]

[1] The writer hopes to deal in a later paper with the general subject of "compound" leaves among monocotyledons, and to discuss the part which chorisis may have played in their origin.

It is unnecessary to follow the relations of the vascular system of the petiole and tendril of *Smilax* in detail, since this has already been done by QUEVA. The general equivalence of the vascular structure in these organs is shown in fig. 3 *B*. It may perhaps be objected that the occurrence of a ring of bundles in the *Smilax* tendril, instead of being an indication of homology with the petiole, may merely represent that skeletal arrangement which best enables the tendril to perform its special function. The weight of this criticism, however, is lessened when we realize that in the leaf tendrils of another member of the Liliaceae (*Gloriosa superba* L., fig. 6), which approach radial symmetry in their external form quite as closely as do the tendrils of *Smilax*, the plan of the vascular system remains purely dorsiventral, although the amount of xylem increases considerably in passing from the lamina to the tendril (figs. 7, 8). The leaf tendrils of *Fritillaria verticillata* Ledebour, and of *Polygonatum cirrhifolium* Royle, resemble that of *Gloriosa* in structure.

According to the phyllode theory advocated by the writer (1), the blade of *Smilax* is not equivalent to the lamina of a dicotyledon, but is merely a "pseudo-lamina" representing an expansion of the upper region of the petiole. The thickened tip (fig. 5 *ap*) which characterizes the blade of some members of the genus, for example *S. mauritanica* Poir., is possibly the last relic of the unexpanded petiolar apex. Each tendril on this interpretation is equivalent to the *petiole+pseudo-lamina*. On the other hand, in *Gloriosa* the leaf seems to be reduced to the sheath or leaf-base alone, the tendril representing the specialized tip of this leaf-base.

Petiolar tendrils, with the blade entirely, or almost entirely, aborted, are not unknown among dicotyledons, as, for example, the first leaves of a species of *Tropaeolum* described by DARWIN (3). In connection with *Smilax*, it is perhaps significant that a tendency to torsion of the petiole or leaf-base is by no means rare among monocotyledons. Cases are known, for instance, in the Commelinaceae, Amaryllidaceae, Zingiberaceae, and Gramineae, as well as in the Liliaceae, to which *Smilax* belongs (6). In most of these instances the torsion, even in the extremest examples, merely leads to resupination of the leaf; but in *Gloriosa* this power

of twisting of a region which, although now apical, seems in reality to be part of the leaf-base, has been utilized in tendril formation. It is interesting that *Polygonatum* includes both species with twisted leaves and also species whose leaves have tendril apices resembling those of *Gloriosa*. Similarly in *Smilax* the torsion capacity of the petiole seems to have fulfilled itself on specialized lines in the production of a climbing organ.

It may be noted in passing that the blade of *Smilax* often conforms to a shape which is one of the most characteristic among those adopted by the pseudo-lamina of monocotyledons, its outline being entire and more or less ovate, with a base which is cordate or somewhat sagittate (fig. 1). Forms of this type, as has been pointed out in a previous paper (1), are not only known in the Liliaceae, but recur in the Alismaceae, Pontederiaceae, Dioscoreaceae, Araceae, and Orchidaceae. This list may now be increased by the addition of *Cyanastrum cordifolium* Oliver, of the Haemodoraceae, and certain species of *Commelina* among the Commelinaceae.

To the writer, the whole organization of the leaf of *Smilax* is best explained according to the phyllode theory, and the interpretation suggested has been reached from this standpoint. At the same time, however, the view here propounded, of the origin of the tendrils of this genus through chorisis of the petiole, in no way depends upon this general theory. There is nothing to prevent its independent acceptance by those who do not share the conviction that the leaves of monocotyledons are invariably of the nature of petiolar or leaf-base phyllodes.

Summary

In this paper the conflicting views hitherto held regarding the nature of the tendrils of *Smilax* are briefly considered, and it is concluded, on grounds of anatomy and external morphology, that these tendrils are equivalent to the petiole in morphological value, and have arisen through chorisis or dédoublement of that organ.

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Cambridge, England

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3. DARWIN, C., The movements and habits of climbing plants. London. 1891.
4. DOMIN, K., Morphologische und phylogenetische Studien über die Stipularbildungen. Ann. Jard. Bot. Buitenzorg **24**:117–326. *pls. 23–33*. 1911.
5. GLÜCK, H., Die Stipulargebilde der Monokotyledonen. Verhandl. Naturhist.-Med. Vereins Heidelberg. N.F. **7**:1–96. *pls. 1–5*. 1901.
6. HANSGIRG, A., Phyllobiologie. Leipzig. 1903.
7. QUEVA, C., Recherches sur l'anatomie de l'appareil végétatif des Taccacées et des Dioscorées. Mém. Soc. Sci. Lille. pp. 457. *pls. 18*. 1894.

EXPLANATION OF PLATE XXII

In all sections xylem is indicated in black, and phloem and undifferentiated bundles in white.

FIG. 1.—*Smilax herbacea* L.: single leaf showing sheath (*s*), tendrils (*t*), and petiole (*p*); $\times\frac{2}{3}$.

FIG. 2.—*Smilax herbacea* L.: *A*, transverse section of very young lamina and accompanying tendrils; vascular tissue too young to be well differentiated; petiole as yet undeveloped; *B*, actual attachment of tendrils; these sections illustrate large size of tendrils relative to lamina in embryonic stage; $\times 31$ circa.

FIG. 3.—*Smilax herbacea* L.: transverse sections of mature leaf; *A*, through leaf sheath; *B*, through petiole and tendrils, showing relative orientation of organs; $\times 19$ circa.

FIG. 4.—*Smilax herbacea* L.: transverse section close to base of lamina passing through midrib (*mr*) and main lateral (*ml*); $\times 19$ circa.

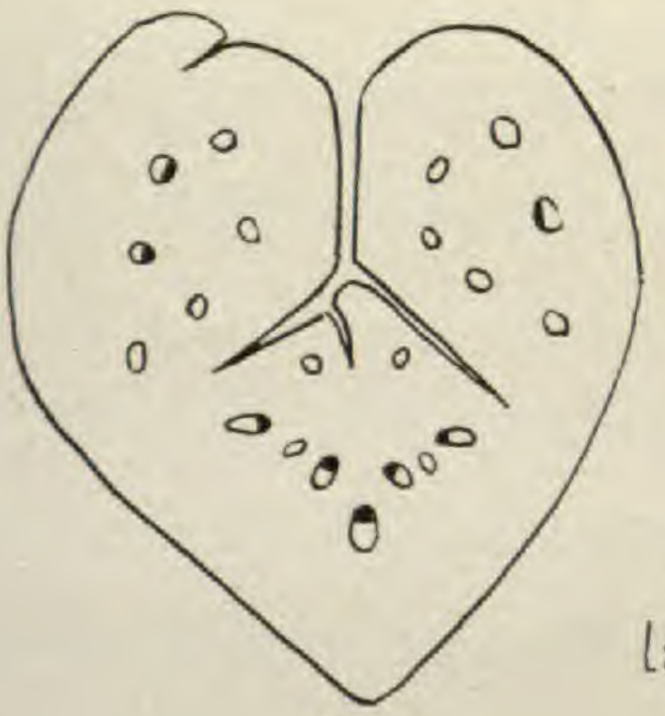
FIG. 5.—*Smilax mauritanica* Poir.: *A*, pseudo-lamina with thickened apex (*ap*); $\times\frac{2}{3}$; *B*, transverse section through apex; $\times 19$ circa.

FIG. 6.—*Gloriosa superba* L.: leaf with apical tendril; $\times\frac{2}{3}$.

FIG. 7.—*Gloriosa superba* L.: transverse section through tendril; lateral veins fused so that only midrib and 2 laterals show; $\times 31$ circa.

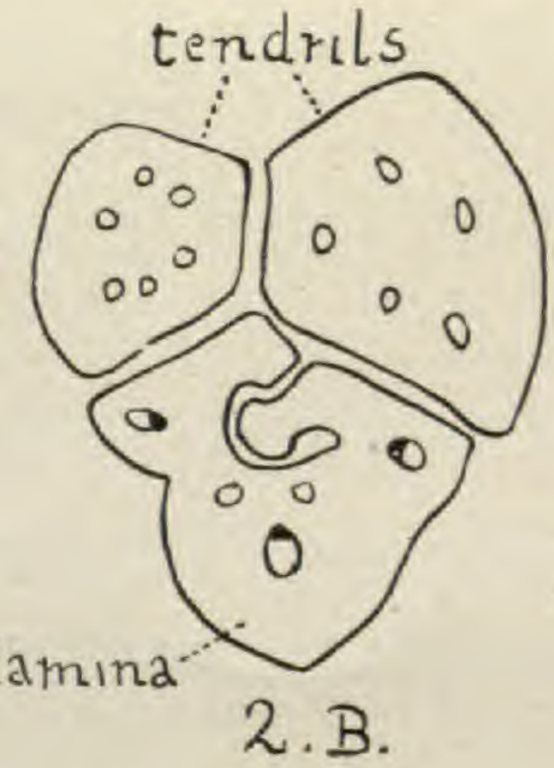
FIG. 8.—*Gloriosa superba* L.: transverse sections of another leaf showing transition from apex of limb (*A*) to base of tendril (*C*); $\times 31$ circa.

SMILAX

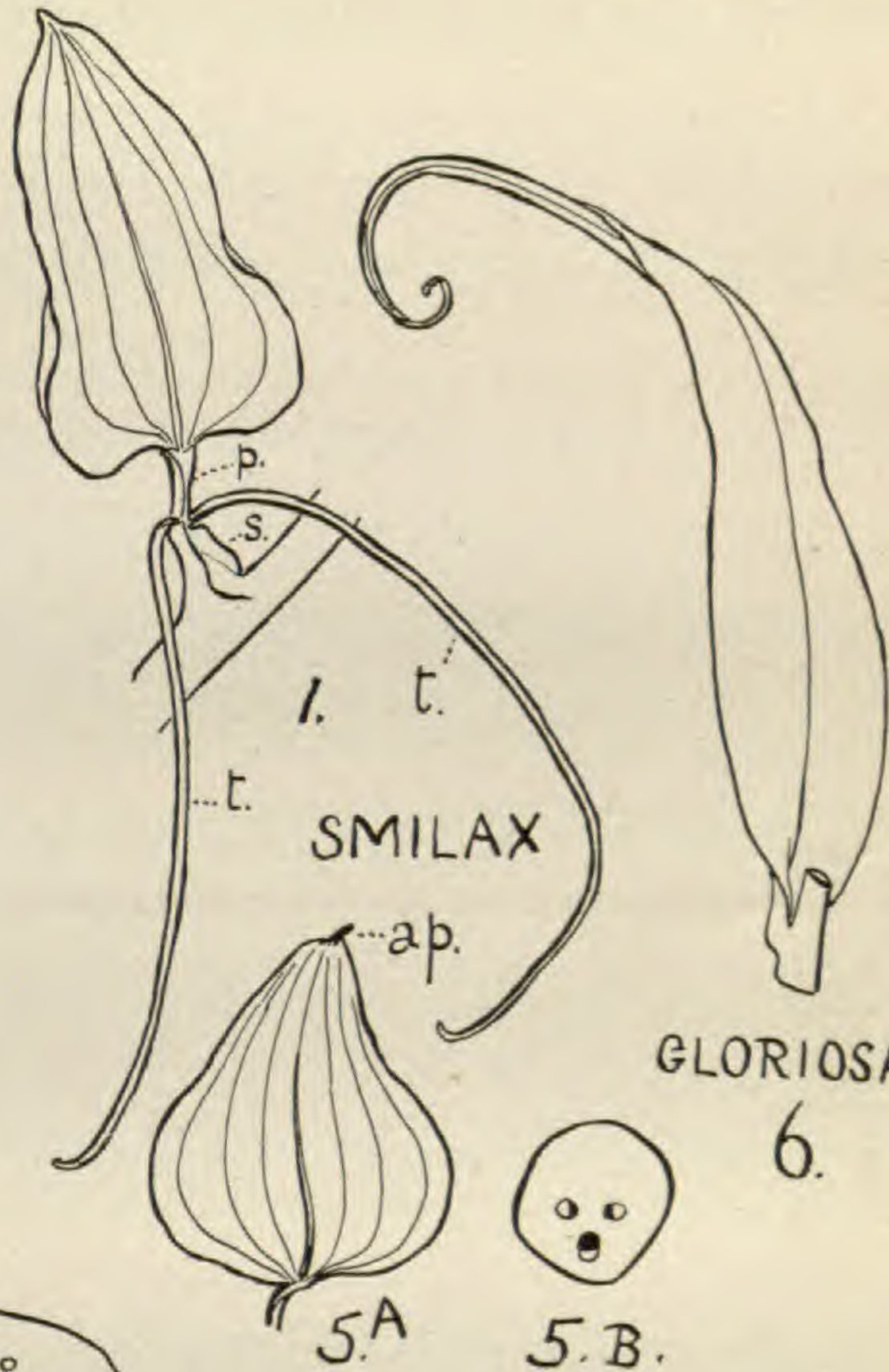


2.A

Embryonic Leaf.



2.B.

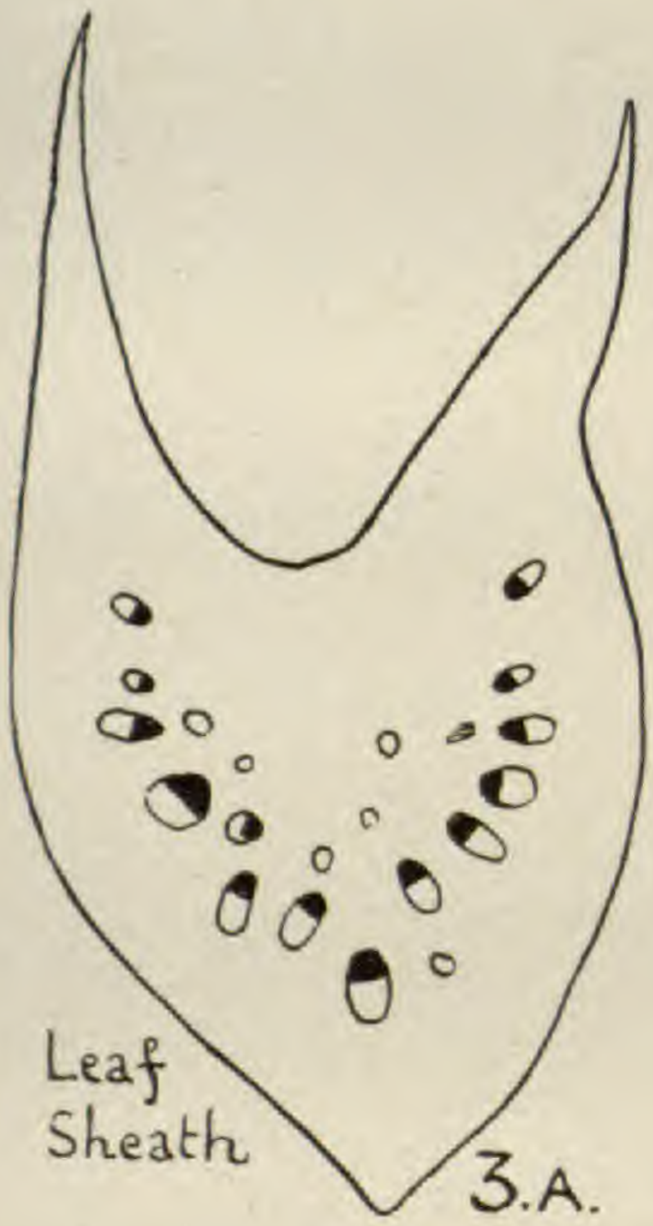


SMILAX

GLORIOSA

6.

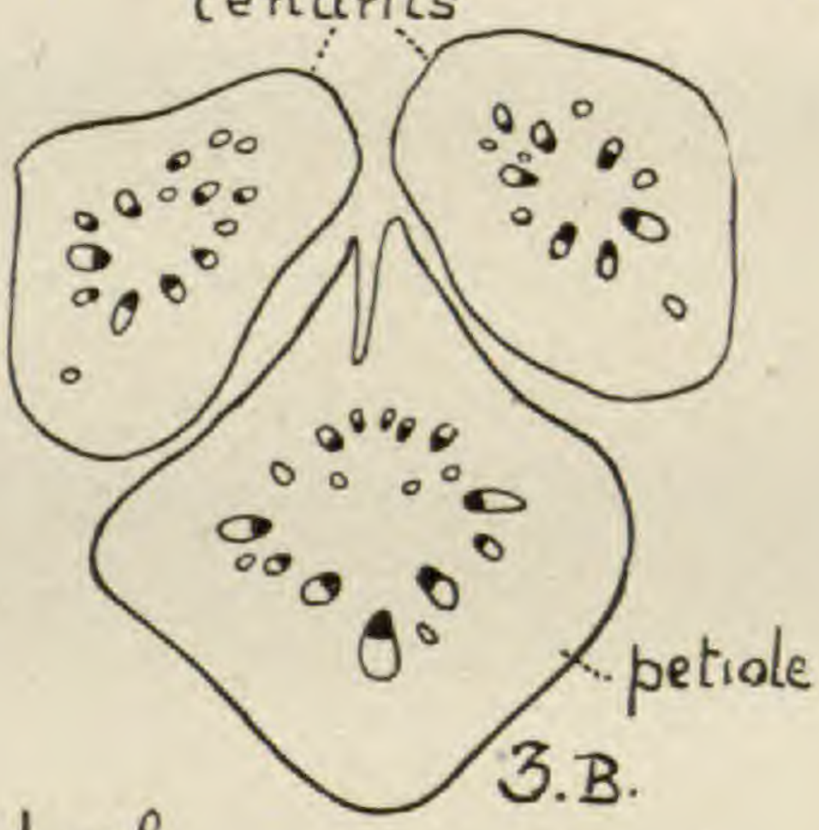
5.A 5.B.



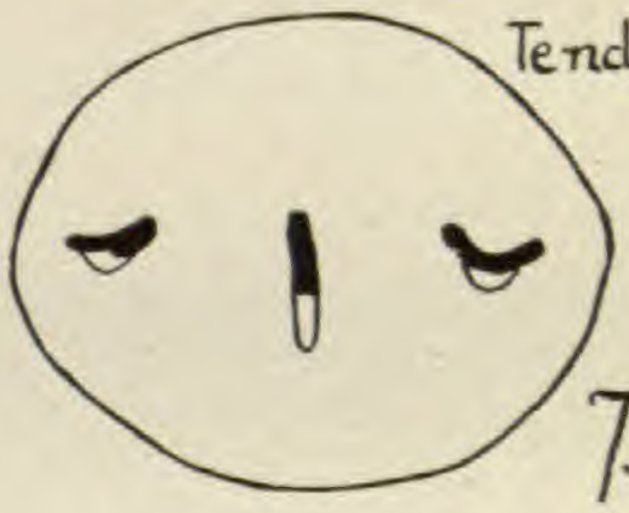
SMILAX

3.A.

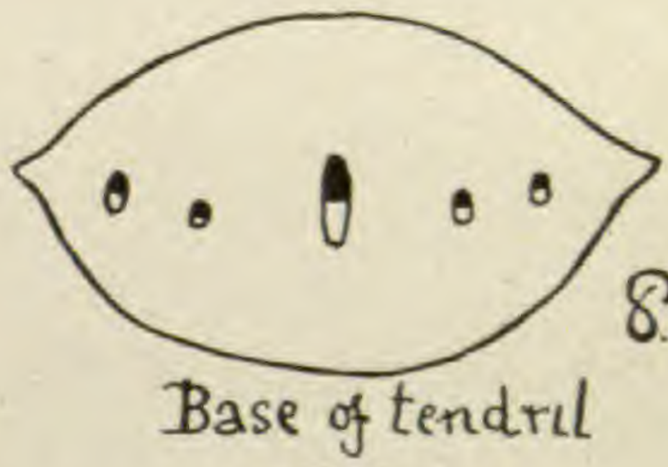
Mature Leaf.



3.B.



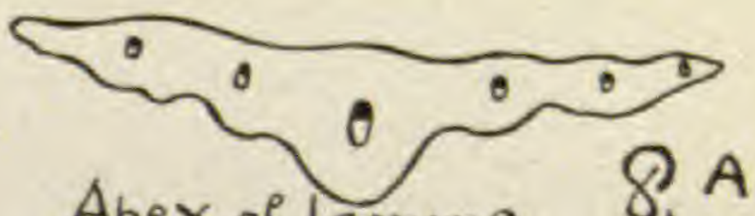
7.



8.C.

Base of tendril

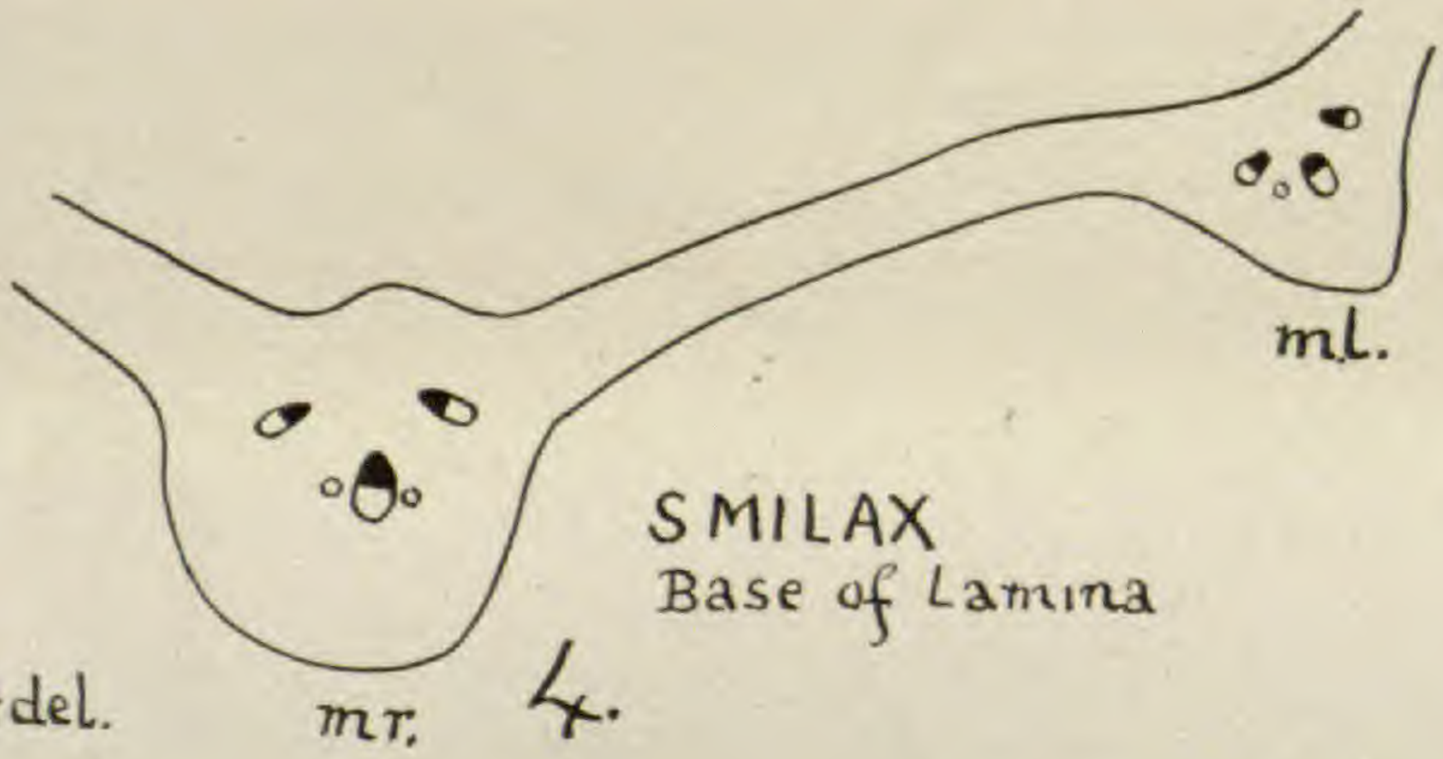
8.B



8.A

Apex of lamina

GLORIOSA



Adel. 4.

SMILAX

Base of Lamina

CURRENT LITERATURE

MINOR NOTICES

Engler's Syllabus.—The seventh edition of Engler's *Syllabus*[1] had marked a great progress beyond the sixth; 135 pages of text had been added and the usefulness of the book was greatly increased by the introduction of illustrations. No such radical changes were made in favor of the eighth edition, which appeared in the summer of 1919. The number of illustrations remained the same, although some were replaced by new ones, and the text was only increased by 11 pages. A few groups, for instance the gymnosperms, show considerable changes. Here the fossil families have received more attention. The Cycadofilicales, which were not mentioned in the seventh edition, are now discussed. Also the Filicales are rearranged and are now headed by the Marattiales. Comparatively few changes are made in the angiosperms. The chapters on plant classification and the outline of floral regions are rewritten by ENGLER himself, and the new literature finds consideration throughout the book. Probably ENGLER and GILG did the best they could while handicapped by lack of literature from English speaking countries.

The rapid growth of the *Syllabus* from edition to edition has undoubtedly suffered a setback through the war. Nevertheless, the book is still the most convenient outline of the generally accepted system which bears ENGLER'S name, and as a very handy index to his *Natürliche Pflanzenfamilien* it remains indispensable to the student of botany. It might be added that its price for American purchasers is about 150 marks.—A. C. NOÉ.

A dictionary of botany.—SCHNEIDER and LINSBAUER'S[2] dictionary of botany lists about 7000 technical terms which are accompanied by short definitions and cross-references, or by articles of sometimes considerable length. SCHNEIDER had published the first edition with the help of a large staff of collaborators, and the second edition was left to the care of LINSBAUER, who is HABERLANDT'S successor in Graz (Austria). The text is preceded by a short bibliography of botanical treatises and periodicals. Only German books are listed, and the *Annals of Botany* and the BOTANICAL GAZETTE are the only English and American periodicals which are mentioned. Most illustrations are rather old and well known to all students of botany. Modern plant morphology is largely neglected, except that the embryology

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[1] ENGLER, ADOLF, and GILG, ERNST, Syllabus der Pflanzenfamilien. 8. Auflage. 8vo. pp. XXXV+395. *figs. 457*. Berlin. 1919.

[2] SCHNEIDER, C. K., and LINSBAUER, KARL, Handwörterbuch der Botanik. 2. Auflage. 8vo. pp. xxi+824. *figs. 396*. Leipzig. 1917.

of the gymnosperms and angiosperms contains a few illustrations from articles by CHAMBERLAIN and Miss PACE which had been published in the BOTANICAL GAZETTE. The articles on paleobotany were first written by POTONIÉ, after whose death they were revised by KUBART in Graz. In these articles the work of SEWARD is never mentioned, and SCOTT is hardly considered. The articles on plant genetics are more up-to-date, and best of all are those on physiology and anatomy.

The lack of knowledge with regard to American and English botanical literature is very pronounced in many German publications. The geologists in Germany know more English because they have to read the publications of the U.S. Geological Survey.—A. C. NOÉ.

Origin and development of Compositae.—SMALL[3] has assembled in book form his papers on the origin and development of the Compositae, which appeared in the *New Phytologist* during 1917-1919. It is an application of our knowledge of evolution, heredity, and geographical distribution to the immense amount of material presented by this great family. This study has been carried on for ten years, and "in so far as success has been achieved in the unravelling of the tangled skein of descent of this particularly large group of angiosperms, it is due to the recognition of new criteria and the critical revision of the old criteria."—J. M. C.

Joseph Dalton Hooker.—BOWER[4] has written a most interesting account of Sir JOSEPH DALTON HOOKER for the series of booklets issued by the "Society for Promoting Christian Knowledge," under the general title "Pioneers of Progress," and the subtitle "Men of Science." The titles of the brief chapters suggest the contents: Birth and education; Foreign travel; Kew; Authorship; The species question; Personal characteristics; Hooker's position as a man of science. The author and his style form a combination that insures authoritative statements and interesting presentation.—J. M. C.

Flowering plants and ferns.—Under this title WILLIS published the first edition of his manual and dictionary in 1897. A second edition appeared in 1904, a third in 1908, and now a fourth edition has been published.[5] The purpose is to supply a convenient summary of information about plants, and the result is a very convenient book of reference. The most noteworthy feature of the present edition is the incorporation of all the parts of the earlier editions

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[3] SMALL, JAMES, The origin and development of the Compositae. pp. xi+334. *pls. 6*. London: William Wesley & Son. 1919.

[4] BOWER, F. O., Joseph Dalton Hooker. pp. 57. New York: Macmillan Co. 1919.

[5] WILLIS, J. C., A dictionary of the flowering plants and ferns. Fourth edition. pp. xii+712+liv. Cambridge: The University Press. 1919. New York: G. P. Putnam's Sons.

into one general dictionary, and the omission of the former part dealing with morphology, classification, geographical distribution, economic uses, etc. There are approximately 20,000 entries, so that a wealth of information is available at a minimum of trouble.—J. M. C.

NOTES FOR STUDENTS

Tropical rain forests.—Two recent contributions have added considerably to our knowledge of the ecology of tropical rain forests, especially as both present quantitative data concerning habitat and also the vegetation. The first by BROWN[6] deals with the vegetation of a portion of the Philippine Islands, and the second by MCLEAN[7] with some of the rain forest near Rio de Janeiro, Brazil, and is the continuation of a report already noticed.[8] This rain forest of Brazil is regarded as the climax type for a large portion of the country. A biological spectrum of the Raunkiaer type would show an enormous preponderance of woody plants arranged in three distinct strata, the ground flora being comparatively open. There is a great diversity of species, with the Leguminosae as the most prominent family, and the Rubiaceae and Piperaceae important among the undershrubs. Ferns and lycopods are largely limited to rocky spots. Conspicuous flowers are abundant in the upper canopy and notably lacking below. Buttressed tree trunks are rare, in spite of the frequency of violent winds, but thorny stems are frequent even in large trees. The floristic diversity and the contrasting uniformity of appearance, especially in leaf form, are ascribed to (1) the antiquity of prevailing conditions, and (2) the peculiarity of the environment. The soil is shallow and pervious, with a water-holding capacity of about 40 per cent, and an average water content of 10 per cent. It is deficient in mineral nutrient material and particularly in calcium carbonate. The humus content is about 3 per cent. *Mycorhiza* are very abundant. A very considerable amount of rain is intercepted by foliage and evaporated into the air, thus reducing the rainfall efficiency. Light measurements made with photographic exposure meters show the average ratio of the light outside and that within the deep forest to be 1:0.06; some spectroscopic measurements, however, tend to show that the photosynthetic efficiency of the shade illumination is relatively greater than the actinic.

The leaves of the forest are in general characterized by their larger size, the small number per plant, and the frequency of nyctitropic movements and of the vertical position. The shade leaves show conspicuous water-storing epidermis, reduced and undifferentiated mesophyll, and occasional epidermal

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[6] BROWN, W. H., Vegetation of the Philippine Mountains. Dept. Agric. and Nat. Resources, Bur. Sci. Pub. **13**:434. *pls. 41. figs. 30.* Manila. 1919.

[7] MCLEAN, R. C., Studies in the ecology of tropical rain forest; with special reference to the forests of South Brazil. Jour. Ecol. **7**:121–172. *figs. 10.* 1919.

[8] BOT. GAZ. **69**:92–94. 1920.

papillae. The leaf area of the sun foliage is approximately the same as that of the shade leaves, but the latter are decidedly larger and narrower. Red coloration is common in the young shade leaves, and such leaves are shown to have a higher rate of respiration. The percentage of carbon dioxide within the forest is shown to be high, and here light is doubtless the limiting factor of photosynthesis.

BROWN agrees with MCLEAN in recognizing valley and mountain forest types, and in addition describes a distinct transition form. The lowland type of the part of the Philippines under consideration is the Dipterocarp forest and extends to an altitude of 600 m. It exhibits distinct strata or stories composed of vegetation 40, 20, and 10 m. high respectively. Epiphytes are largely phanerogams, and are confined chiefly to the largest branches of the tallest trees. Buttressed trees and cauliflora are developed by many species, while the ground covering is characterized by rattans in the rosette stage. On a typical plot there were 22 first story species, 43 second story species, and 23 lower story species.

The midmountain forest extends from 600 m. to an elevation of about 900 m. and shows two stories of about 18 and 8 m. in height. One typical association is termed the *Quercus-Niolitsea* forest from the most abundant genera. The undergrowth is less dense, but the ground cover of ferns and herbaceous plants is better developed than in the Dipterocarp forest. Epiphytes are also more abundant, and include more cryptogams. Above 900 m. a montane forest is developed, exhibiting a single stratum of vegetation some 10 m. high, and known as the "mossy forest" from the great abundance of mosslike plants.

As developed on Mt. Maquiling this last may be termed a *Cyathea-Astronia* association from the two most prominent genera. The herbaceous ground cover is dense; mosses, filmy and other ferns, *Selaginella*, orchids, and lianas are abundant, many growing as epiphytes. Trees are low and contorted in habit. Statistical analyses are made of all types of forest, the size of the trees as well as their floristic relationship being given. Detailed data regarding rates of growth of trees seem to show that they are proportional to the heights of the various forest types. Stations were located in these forests, and at them measurements were made of environmental factors, including temperature, light, evaporation, rainfall, humidity, soil moisture, and wind velocity. Many data were collected and are presented in tables and graphs. Some of the most interesting conclusions based upon these are: (1) humidity is high at all elevations, and the atmosphere is practically saturated at all times under the montane forest; (2) temperature gradually decreases with rising elevation; (3) evaporation in all forests is much less than in the mesophytic forests of the United States, and decreases rapidly with increasing altitude; (4) there is a pronounced, although not severe, dry season; (5) only at low elevations does there appear to be sufficient decrease in soil moisture during the dry season to have any harmful effect on vegetation; and (6) the increase in her-

baceous vegetation with increasing altitude is due to increased soil moisture and decreased rate of evaporation.

An analysis of the foliage shows that leaves with entire margins are more abundant in the lower stories than in the upper, and at lower elevations than at higher ones. Classified according to the system devised by RAUNKIAER, the plants over a meter in height are found to show but three leaf sizes, and the number of species with microphyll, mesophyll, and macrophyll leaves are, for the most part, Dipterocarp forest, respectively 4: 79: 9; for the mid-mountain forest 4:61: 5; and for the mossy forest 8:8:0, showing a decided decrease in leaf size with increase in elevation.

The Philippine vegetation is made more attractive to the reader by numerous good photographs reproduced on excellent plates.—GEO. D. FULLER.

Transpiration studies.—A series of papers by SAYRE[9] contains some interesting results regarding transpiration from hairy leaves. The leaves of the mullein, *Verbascum Thapsus*, offer more resistance to water loss in darkness than in light, in still air than in wind, and respond rather more to changes in environment than do the smooth leaves of tobacco, *Nicotiana* sp. The removal of the hairs of the mullein leaves resulted in no change of resistance in still air and light, and but slightly reduces resistance in wind and light. There was a greater reduction of resistance to water loss caused by the removal of hairs in still air and darkness, as under such conditions transpiration is entirely cuticular. Hence it appears that, in this plant at least, hairs as a covering affording protection against ordinary intensities of wind and light are quite inefficient and may be disregarded. The stomatal water loss is 20–40 times the cuticular, and only the latter is influenced by the removal of the hairs.

Transpiration, humidity, evaporation, and sunshine were recorded along with the water loss from sealed potted plants. Stomatal transpiration is shown to be governed by various factors which control the opening and closing of the stomatal pores, and by the diffusion gradient. An increasing saturation deficit of the intercellular spaces of the mesophyll is regarded as important in increasing the resistance of the leaf to water loss while stomata are open, but as of no effect after stomata are closed by darkness.

The tobacco and *Verbascum Thapsus* show a rhythm in the transpiration curve in darkness for one day only succeeding a day of normal light exposure, but *V. Blattaria* exhibits no such rhythm under the same conditions.—GEO. D. FULLER.

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[9] SAYRE, J. D., Comparative transpiration of tobacco and mullein. Ohio Jour. Sci. **19**:422–426. *fig. 1.* 1919.

———, Factors controlling variations in the rate of transpiration. *Loc. cit.* **19**:491–509. *figs. 9.* 1919.

———, The relation of hairy leaf coverings to the resistance of leaves to transpiration. *Loc. cit.* **20**:55–75. *fig. 7.* 1920.

A new rubber.—HALL and GOODSPEED,[10] in connection with the war demand, have investigated a possible native source of rubber, studying especially *Chrysothamnus*. They found rubber of high grade in *C. nauseosus*, and adopted the name "chrysil" for this particular kind or rubber. This species includes 22 recognized varieties, which have been taxonomically presented by HALL.[11] Of these varieties, 12 were examined and all found to contain rubber. The shrub grows readily from seed and matures in 6–8 years. The largest stands occur in Colorado, Nevada, and Utah; and it is estimated that the total amount of rubber present in wild shrub is over 300,000,000 lbs.

Chrysil is not a latex rubber, but occurs in individual cells. The results of 180 chemical analyses and 80 microscopical examinations are tabulated. The rubber occurs in the plants in greatest abundance about the soil line, being present in the root in only the upper parts, and occurring in small amounts in young twigs and leaves. The best varieties for rubber are those growing in alkaline soils, so that the culture of this rubber-yielding plant could be developed extensively in alkaline regions unsuitable for other crop plants.

The authors[12] also give an account of the occurrence of rubber in other species of *Chrysothamnus* and in *Haplopappus*. They also append a long list of species, chiefly Compositae, in which no rubber was found.—J. M. C.

Philippine bamboos.—In a recent bulletin BROWN and FISCHER[13] have presented much interesting data regarding the taxonomy, ecology, and economic value of Philippine bamboos. Nine genera, including 30 species, 17 erect and 13 of climbing habit, are described, and a key for identification is provided. Planting and harvesting methods are discussed, and some data regarding market prices given; but of much more interest to ecologists is a series of accurate growth records extending over a period of 20 weeks. Growth rates of 2 m. per week are not uncommon, and several shoots showed a weekly growth in excess of 3 m.—GEO. D. FULLER.

Salix.—SCHNEIDER,[14] in continuation of his studies of American willows, has discussed the section Adenophyllae, recognizing 8 species, one of which (from California) is described as new. The discussions and descriptions are very full, and are accompanied by complete citations of collections, so that taxonomists will be at no loss as to the plants referred to.—J. M. C.

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[10] HALL, H. M., and GOODSPEED, T. H., Chrysil, a new rubber from *Chrysothamnus nauseosus*. Univ. Calif. Publ. Bot. **7**:183–264. *pls. 18–20. figs. 6.* 1919.

[11] HALL, H. M., *Chrysothamnus nauseosus* and its varieties. *Idem* **7**:159–181. 1919.

[12] HALL, H. M., and GOODSPEED, T. H., The occurrence of rubber in certain west American shrubs. *Idem* **7**:265–278. *figs. 2.* 1919.

[13] BROWN, WM. H., and FISCHER, A. F., Philippine bamboos. P.I. Dept. Agric. and Nat. Res., Bur. For. Bull. **15**: pp. 32. *pls. 33.* 1918.

[14] SCHNEIDER, CAMILLO, Notes on American willows. VII. Jour. Arnold Arboretum **1**:147–171. 1920.

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THE
BOTANICAL GAZETTE
JUNE 1920

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, á 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

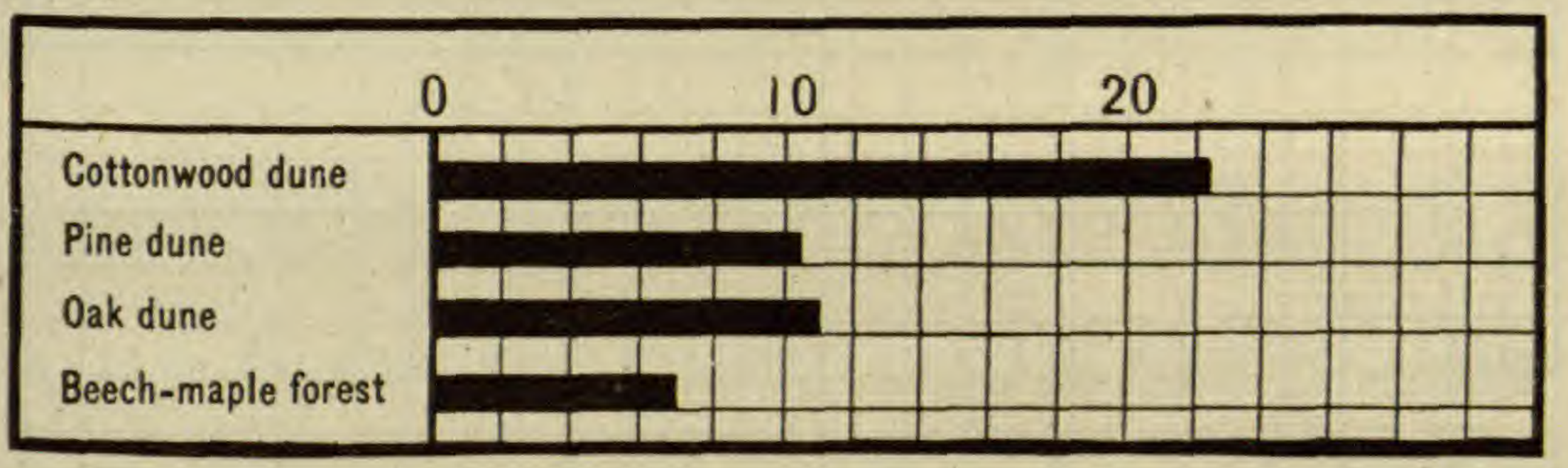

FIG. 1.—Average of mean daily evaporation rates for the 4 associations for seasons 1910, 1911, 1912.

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 a long 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 STRUCTURES OF TAXUS CANADENSIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 267

A. W. DUPLER

(WITH PLATE XXIII AND SIXTY FIGURES)

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.

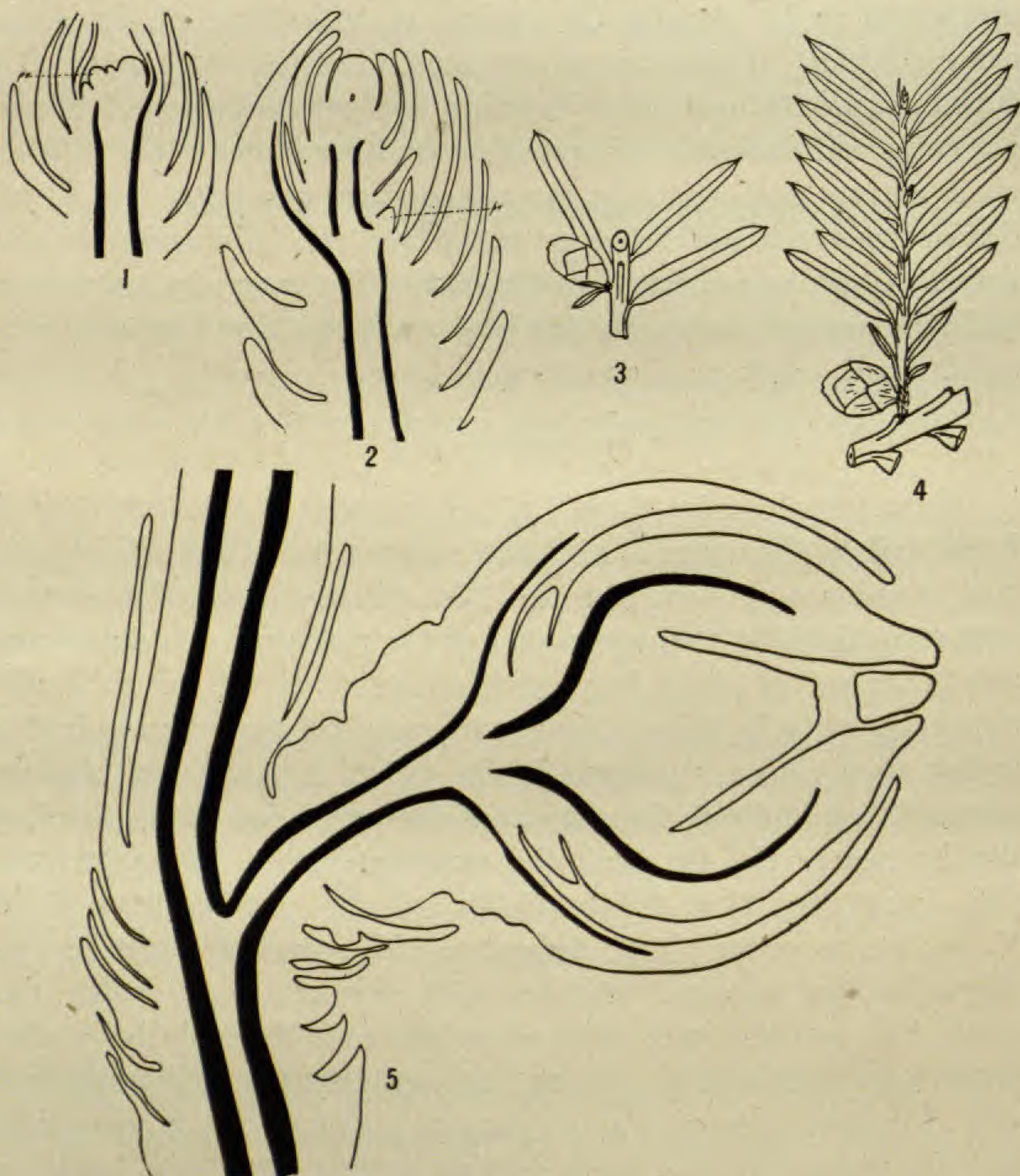
Ovuliferous bud

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

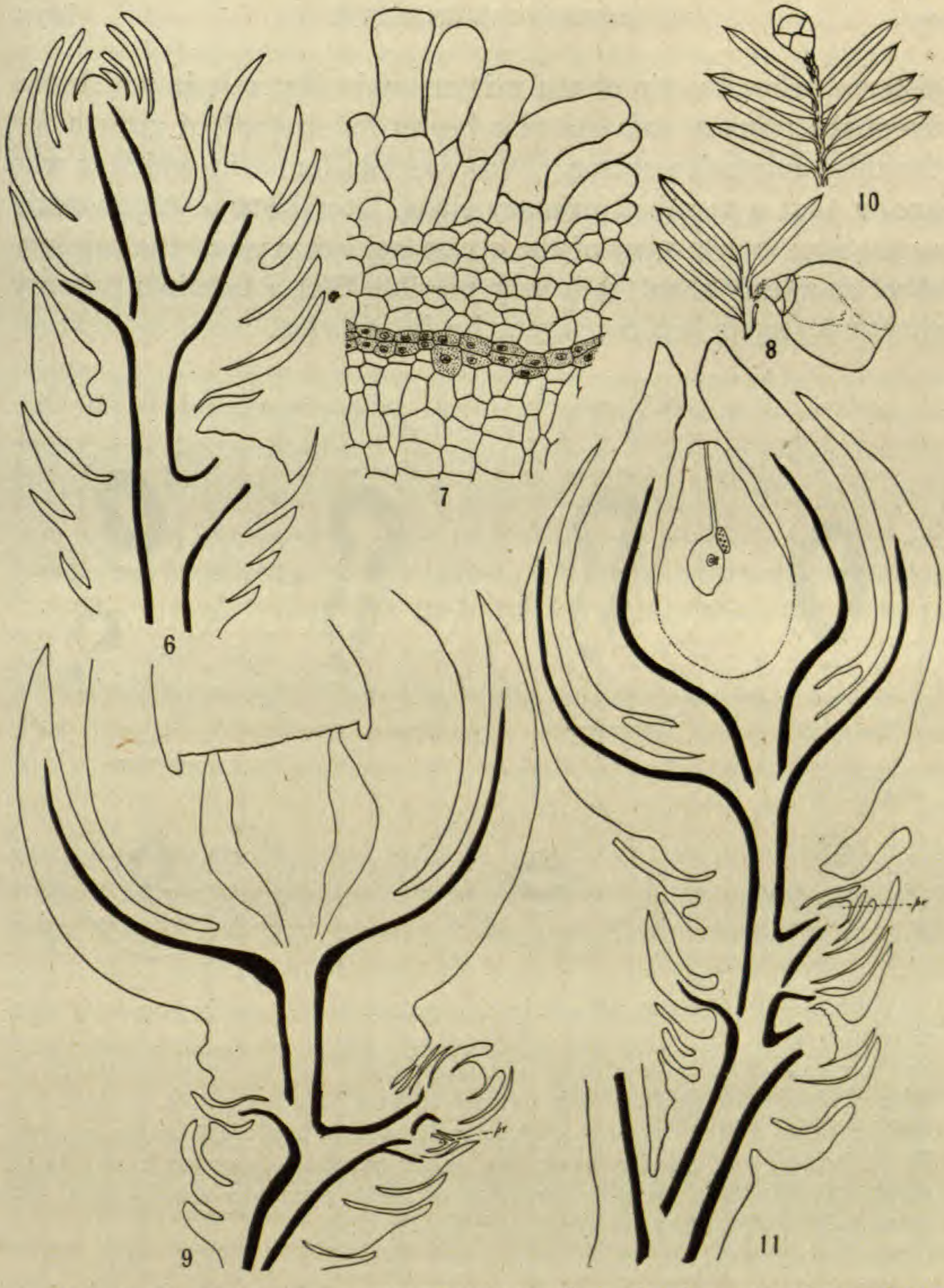
strobilus already described (13), having very thick epidermal walls, especially on the outer surface, stomata on the inner surface, and rather large air spaces. They are brownish and lack chlorophyll.

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

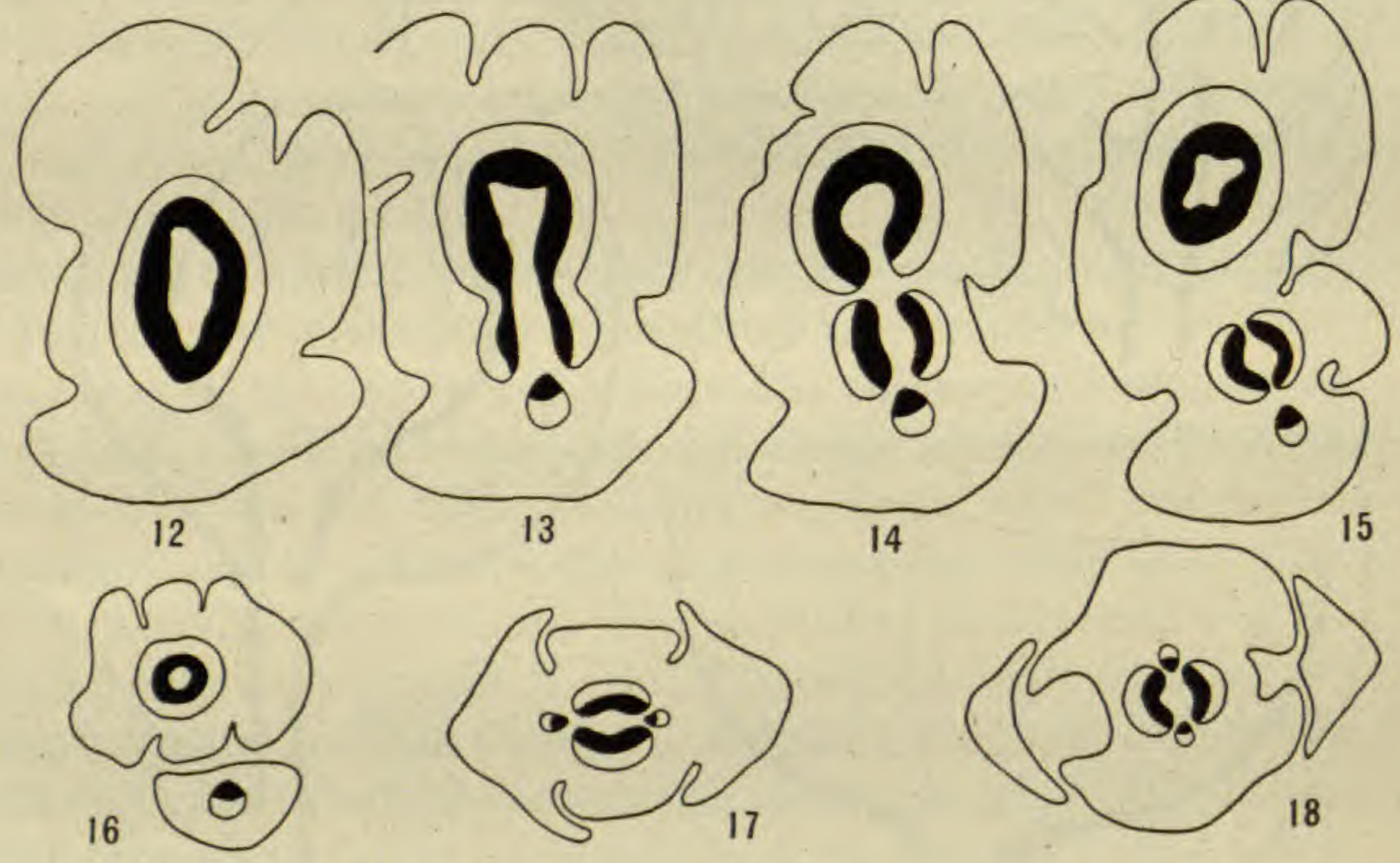
season, and that the maturity of the secondary shoot with the ovule results in the death of the primary shoot as well. This is not the normal situation, as usually only the secondary shoot with the ovule drops from the primary shoot, which remains in the axil of the leaf, a branch scar showing the place 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 absciss 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.

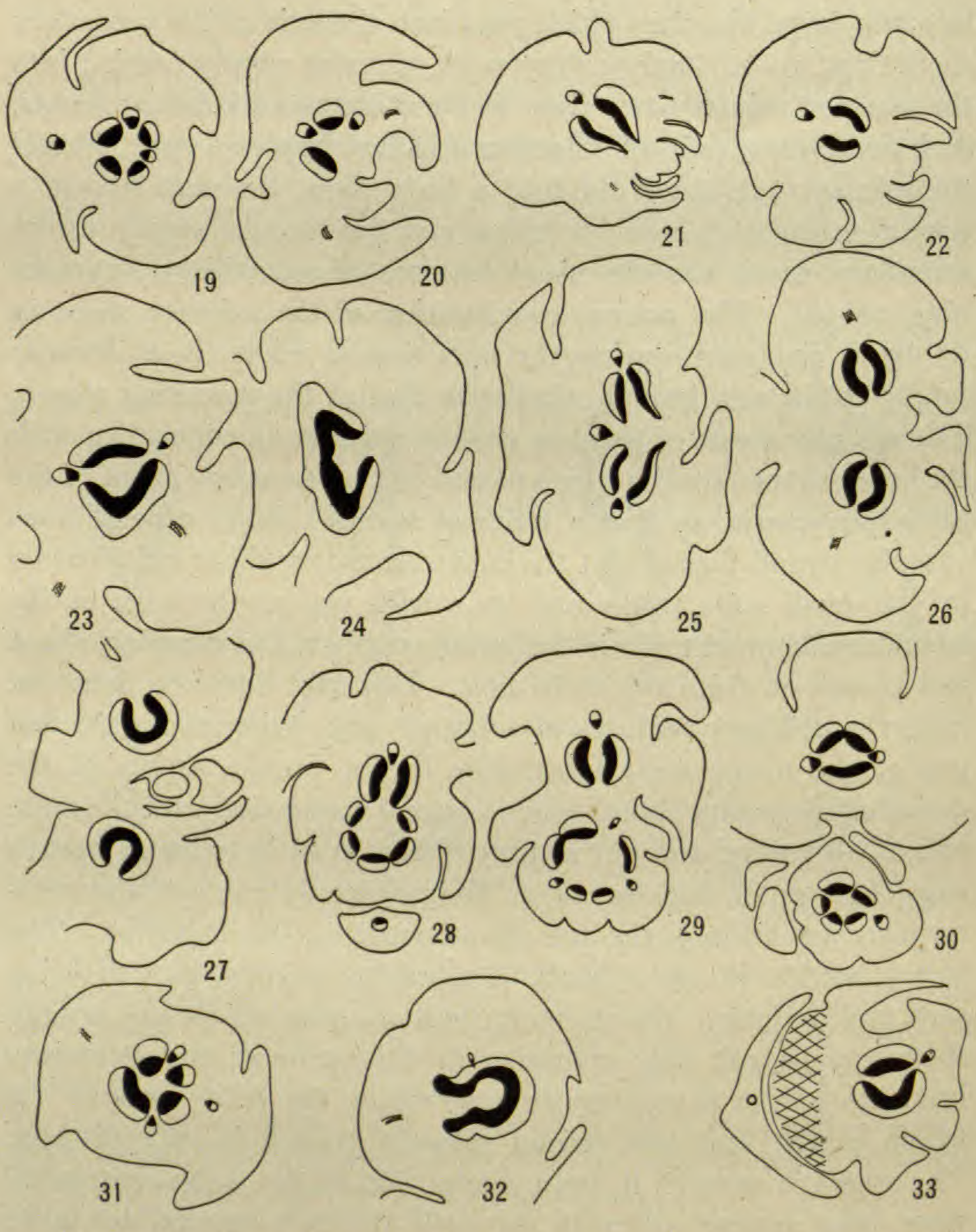


FIGS. 12–18.—Series at different levels showing vascular supply from leafy shoot to primary shoot: fig. 12, vascular cylinder of leafy shoot; fig. 13, trace to fertile leaf and formation of vascular strands to primary shoot; fig. 14, vascular strands for primary shoot separated from main cylinder, showing 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

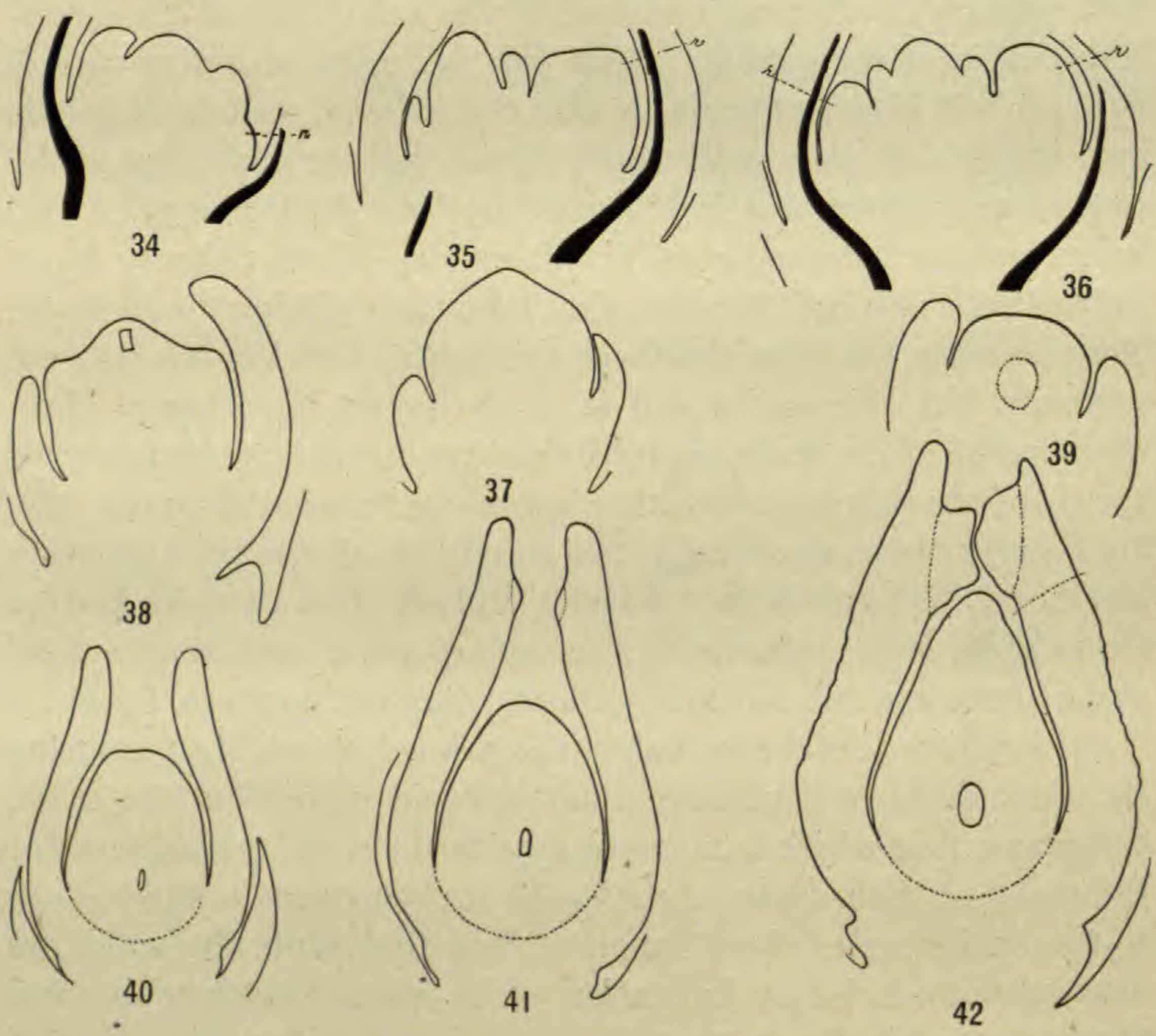
GENERAL FEATURES.—The primordium of the secondary shoot first appears as a lateral structure in the axil of one of the uppermost scales of the primary shoot (fig. 34), soon becoming conical (fig. 35). It is generally stated that the terminal scale is the fertile one, but one or more small scales usually 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

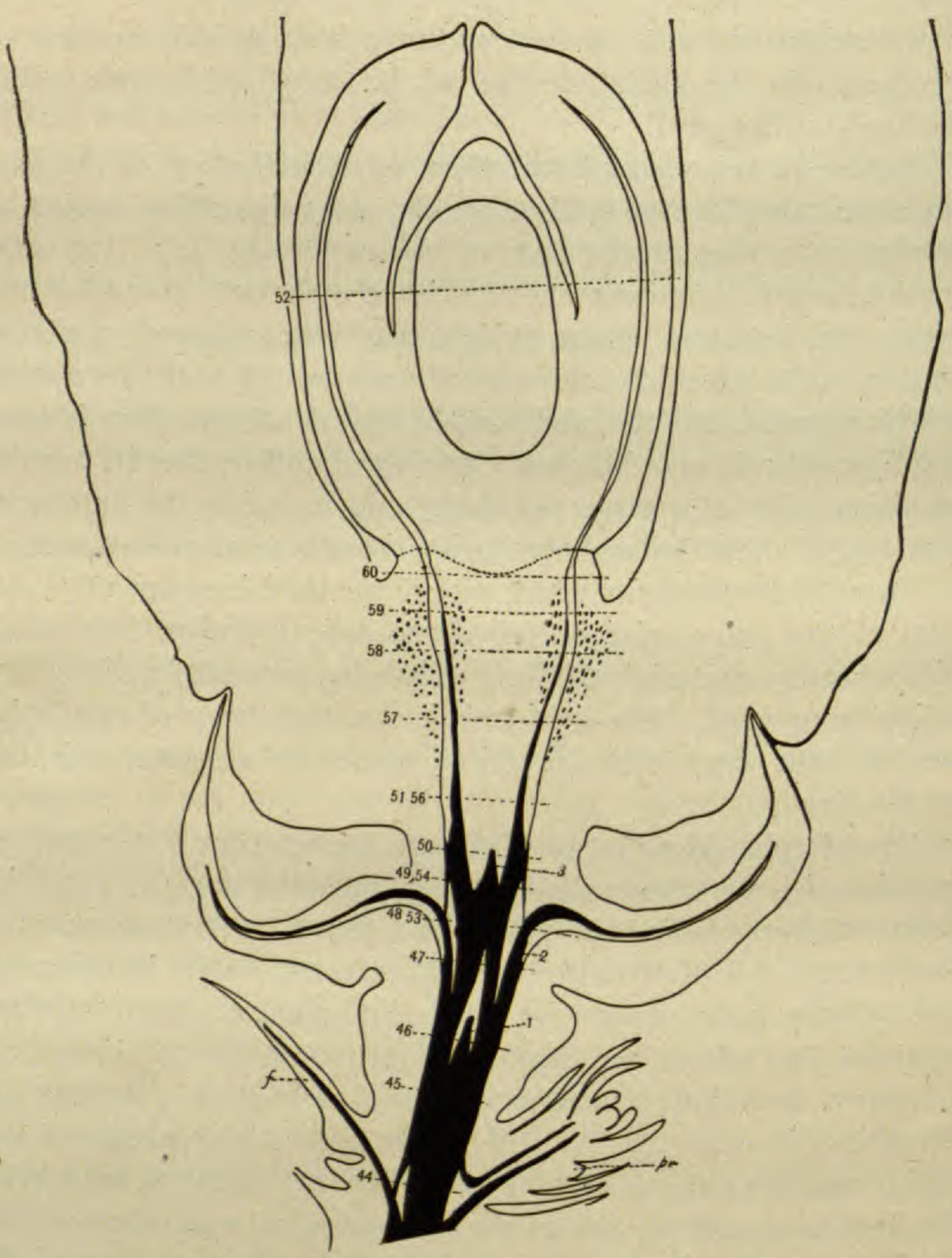


FIG. 43.—Semi-diagrammatic longitudinal section through primary shoot with secondary shoot and portion of mature ovule, ×17; outlines of primary and secondary shoots and aril of ovule made with camera lucida, ovule inserted diagrammatically; outlines of vascular supply also made with camera; note young 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.

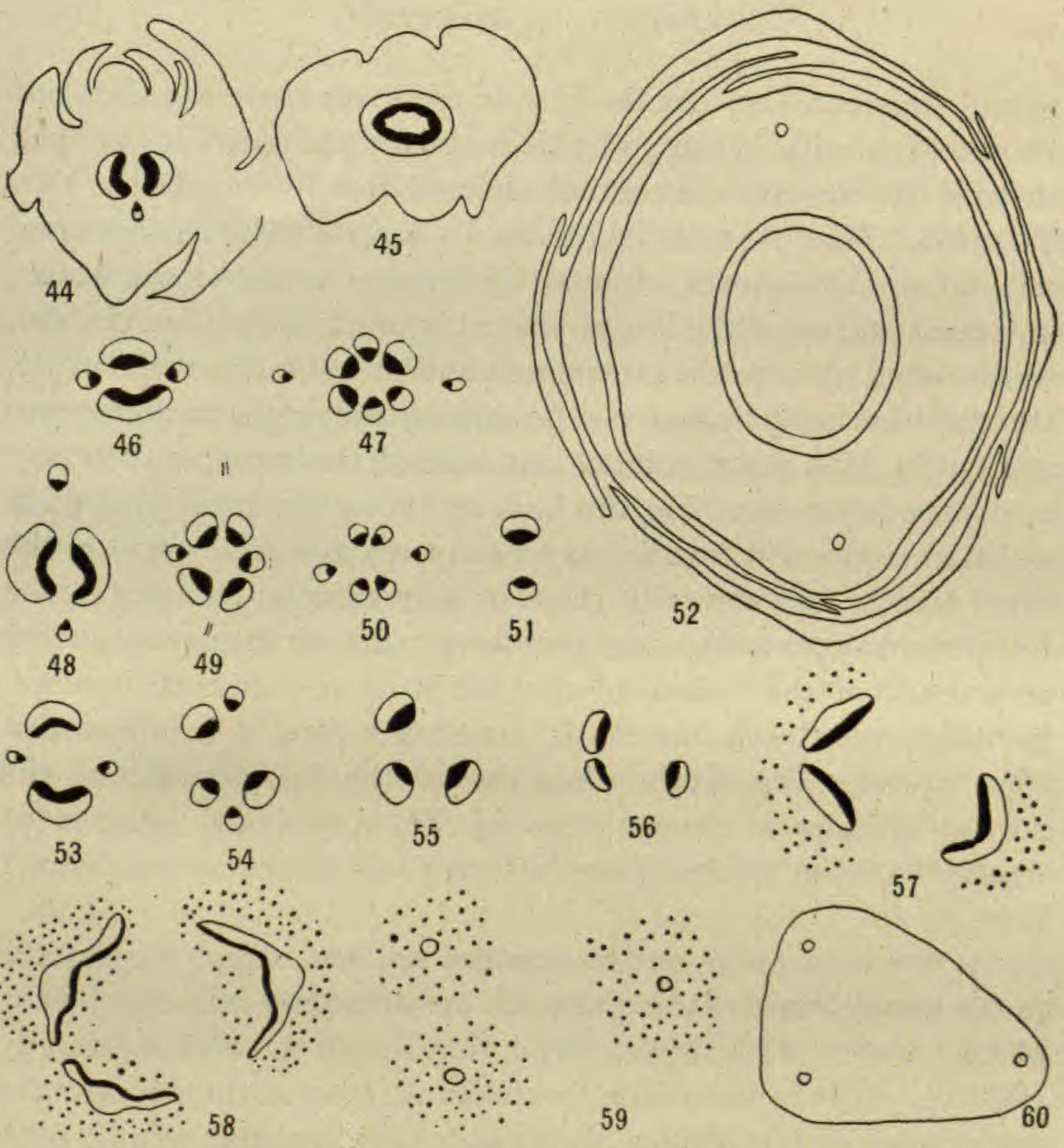
Attempts have been made to relate the taxads to the cycads on account of the fleshy character of the ovule, regarding *Cephalotaxus* and its relatives as bridging from cycads to conifers. The cycadean origin of the conifers does not harmonize with the known facts, however, and the attempt to relate all 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.

The writer acknowledges obligations to Professors JOHN M. COULTER and CHARLES J. CHAMBERLAIN, under whom the study of *Taxus* was begun.

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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.

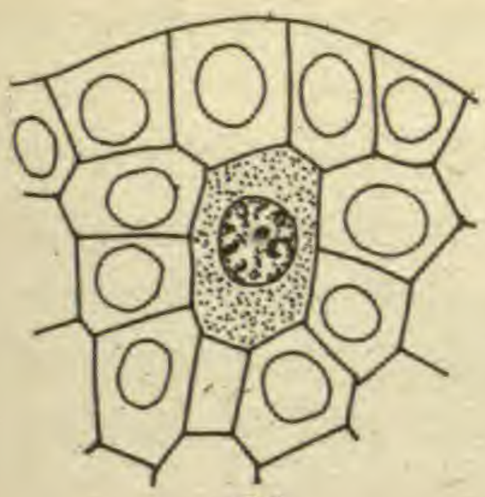
FIG. 65.—Portion of nucellus showing several-layered epidermis (cells without nuclei), megasporangium wall (cells with nuclei), and sporogenous tissue (shaded) with group of megaspores; ×475.

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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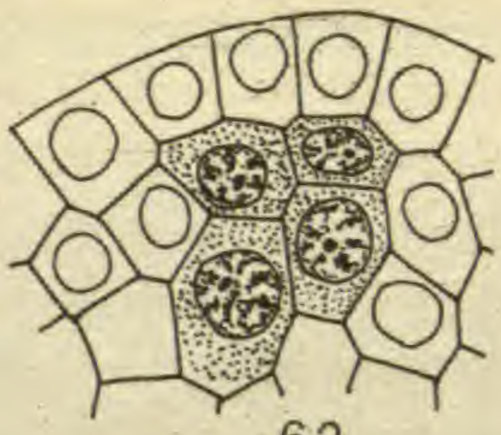

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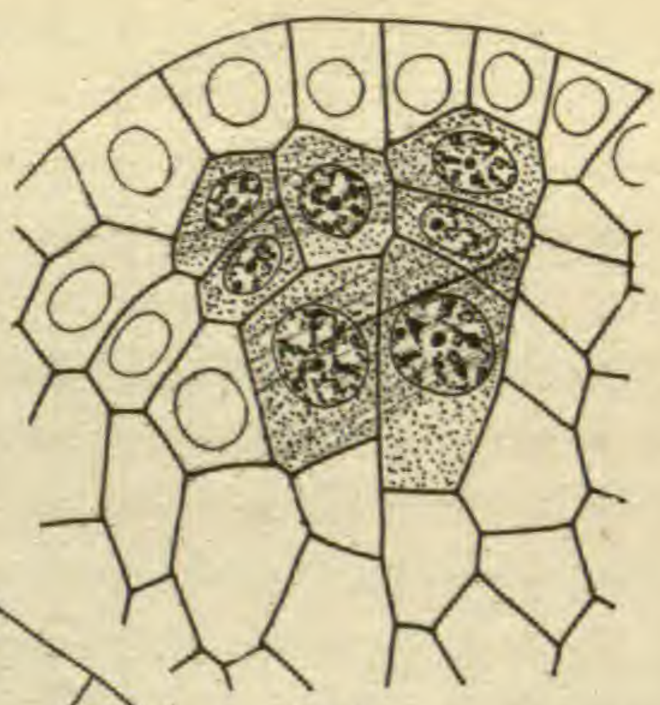

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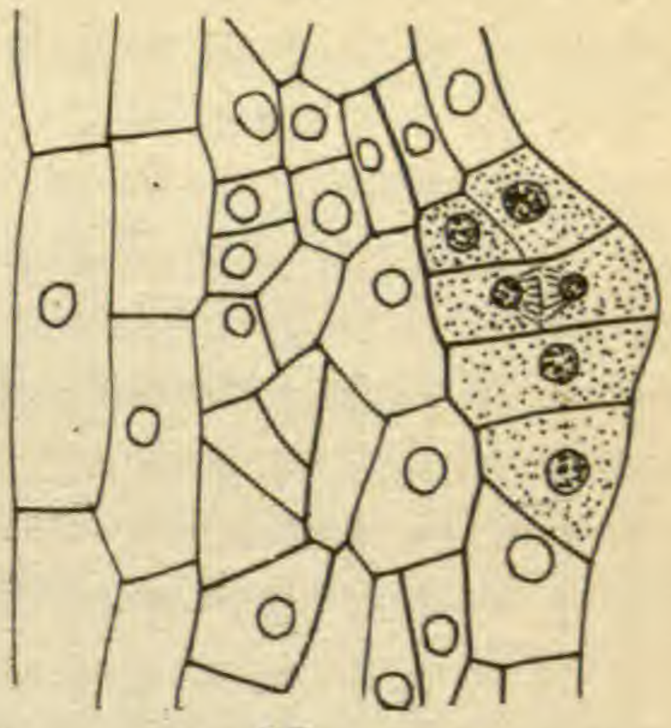

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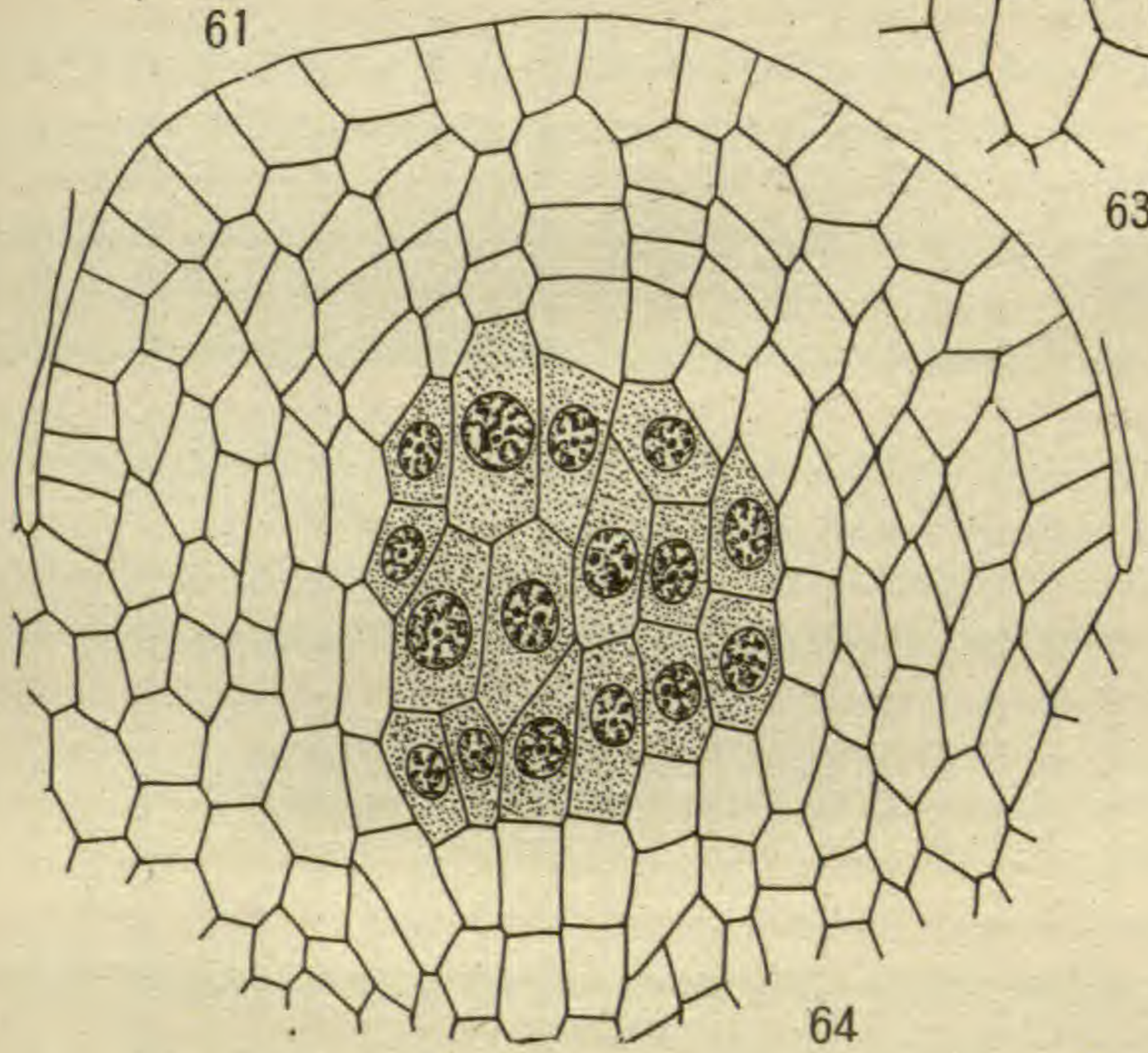

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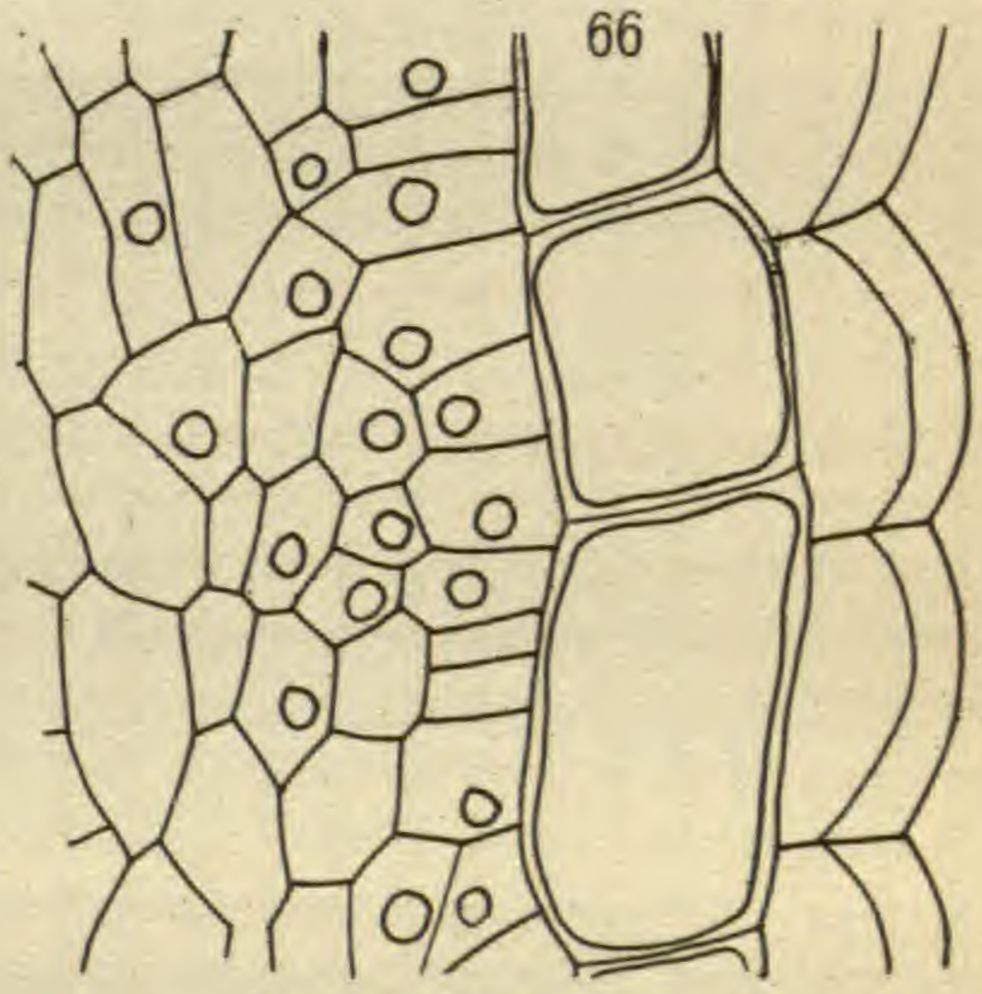

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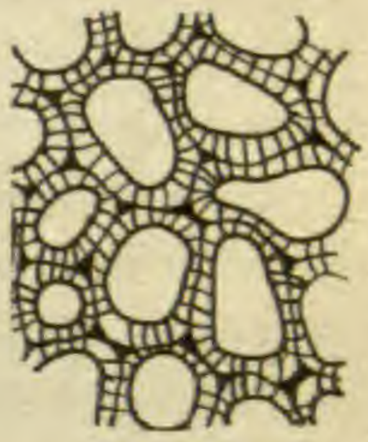

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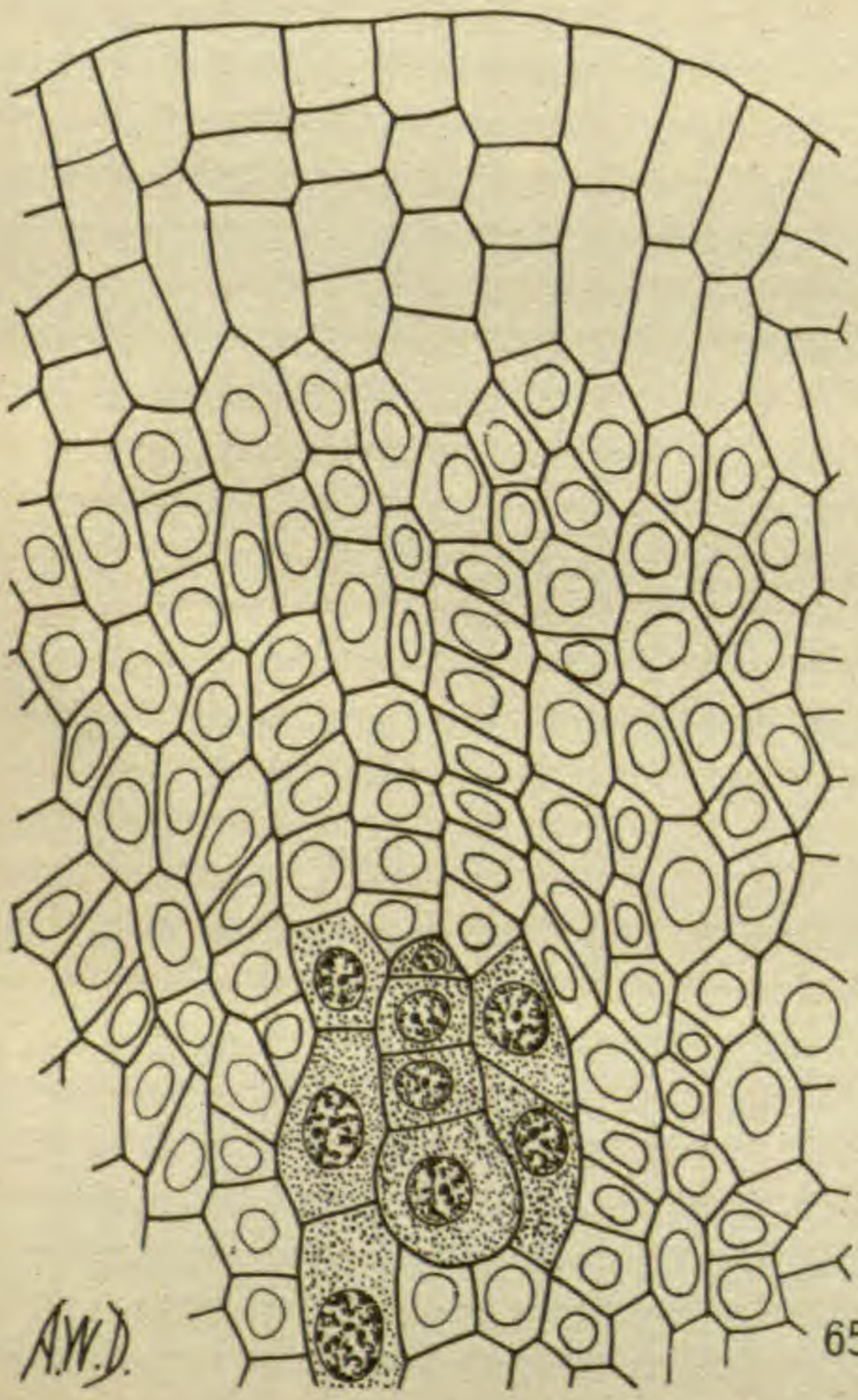

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67

DUPLER on TAXUS

ROT OF DATE FRUIT[1]

J. G. BROWN

(WITH FIVE FIGURES)

In the autumn of 1917, Dr. A. E. VINSON of the Arizona Experiment Station brought to the writer a small box of dates from the Yuma date orchard with the request that the organism with which they were badly infected be determined. The fruits were carefully examined, but it was impossible to give the requested information without further investigation; and it was suggested by Professor THORNBER, Botanist of the Station, that since the problem concerned food conservation it would be especially profitable to attack it at once. The advice was acted upon, and the results are partly set forth in this preliminary paper.

For the purpose of observing the disease in the field, a trip was made to the orchard in December 1917, and a careful inspection of trees and fruit was undertaken. A glance at the figures will show that abundant evidence of disease was not difficult to find. The ground under many of the trees was thickly covered with the spoiled fruit (fig. 1), and numerous clusters still hanging to the trees suggested a severe attack of "plum pockets," for a large percentage of the fruit had become mummified (fig. 2). Some of the fruit on the ground was covered with molds, and similarly infected fruit was found wedged between the leaf bases and tree trunks and on the ground half buried in the soil. Of the several varieties of date palms comprising the orchard, the Deglet Noor appeared to be the favorite host. It was stated that the year had been an especially bad one, about 90–95 per cent of the crop being infected. The fruit was selling at the orchard at 35–45 cents per pound. Since many of the trees produce from 200 to 400 pounds of salable fruit under normal conditions, the loss was considerable.

Both Yuma and Tempe date orchards were affected much less by the rot in 1918 than in 1917. Table I gives precipitation and

[1] Preliminary paper.

FIG. 1.—Deglet Noor variety showing ravages of date rot disease; note mummies still hanging to tree and on ground.

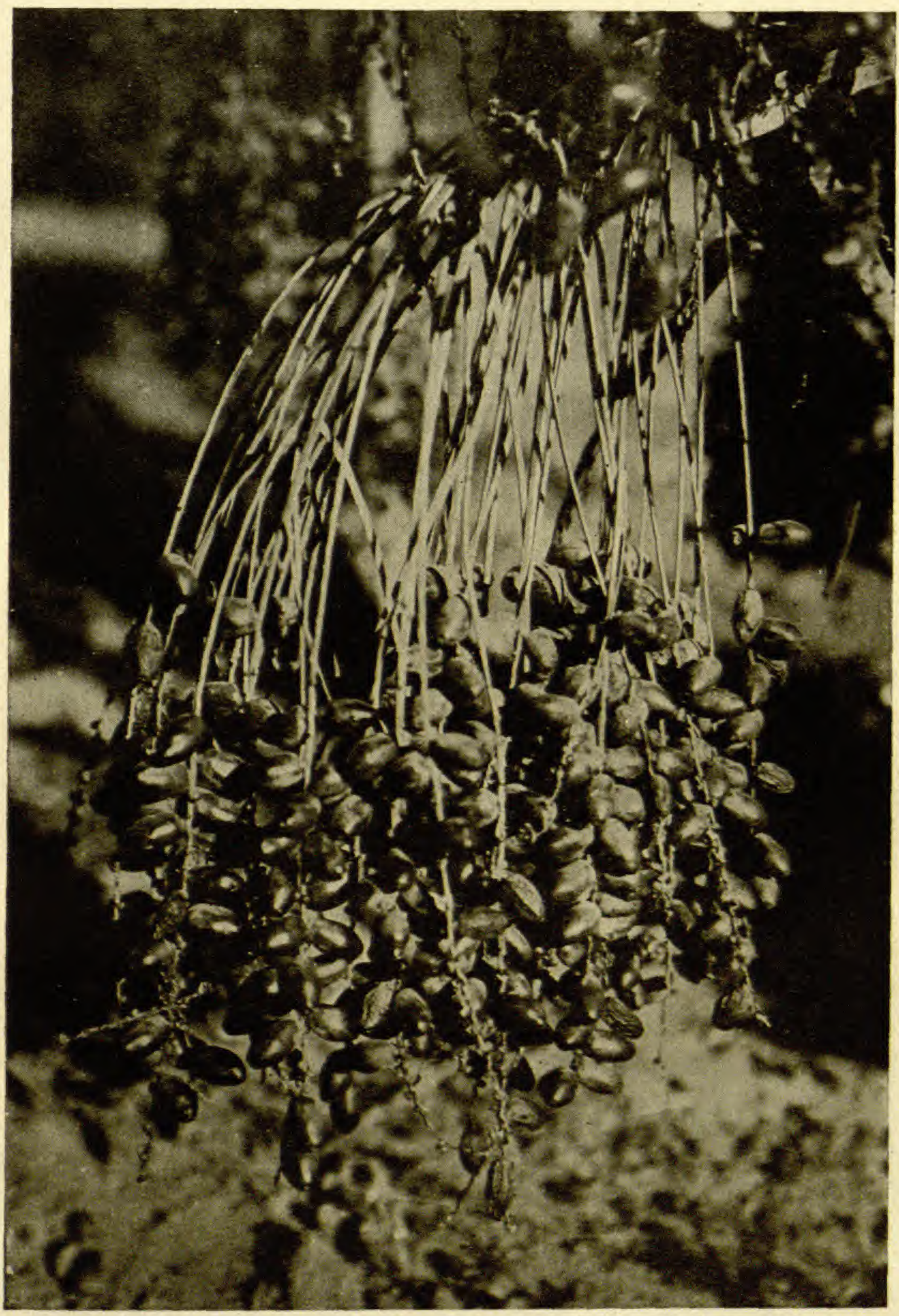

FIG. 2.—Various stages of date rot and mummification

temperature data for the Yuma date orchard covering the two years. Table I suggests that the greater prevalence of the fungi concerned in the rot of the date fruit in 1917 was possibly due to the more favorable conditions of moisture and temperature during April, May, and June, while flowering and fruit setting were in progress. From observations it appears probable that infection occurs at that time. The spring and summer of 1917 had not only an excess of moisture over the same period of 1918, but were also cooler, so that this additional moisture was more effective.

SYMPTOMS.—The fruits showed two main symptoms. Some were flecked with rusty brown spots from the size of a pinhead to areas almost covering one side of the fruit (fig. 4); others showed soft spots varying in size and partly translucent, as though soaked with water or oil (fig. 5). The brown spots gradually increased in size, often coalescing, forming a dark chocolate margined area oval in outline, with depressed, light cream or grayish centers on which clusters of spores finally appeared in pustules (fig. 4, third fruit, third row). The soft spots also enlarged to a similar extent, giving an appearance of rot. In both cases the ruptured epidermis allowed excessive water loss, resulting in the final mummy stage. Mummified fruits sometimes remained for a time in situ, but sooner or later fell to the ground (figs. 1 and 3). The exposed sweet pulp, in the early stages of the soft spots, attracted swarms of small flies and other insects which hovered in and around the fruit clusters, and probably aided materially in carrying the infection.

Examination of the trees revealed numerous brown spots on petioles and ribs of leaves, which also extended down the rhachi of fruit clusters. This suggested a relation between fruit spot and leaf spot, which appears to be confirmed by the laboratory experiments so far completed. In the Tempe date orchard palms three years old already showed the brown spots on the leaf bases.

LABORATORY STUDIES.—Cultures have been made from the spots on leaves, rhachi, and fruits collected in both orchards. The medium used was date agar, prepared according to the method described by SHEAR and STEVENS[2] for prune agar by substituting

[2] SHEAR, C. S., and STEVENS, N. E., Bur. Pl. Ind. Circ. no. 131.

FIG. 3.—Cluster of date fruit from left side of tree shown in fig. 1, showing most of fruit fallen, owing to attack of spot and rot fungi.

TABLE I

	January	February	March	April	May	June	July	August	September	October	November	December
	Comparative precipitation 1917											
Precipitation in inches	.....	.....	00	0.27	0.05	00	0.93	0.24	0.18	00	00	00
Departure from normal	.....	.....	−0.26	+0.20	+0.05	−0.06	+0.59	−0.64	+0.09	−0.24	−0.27	−0.41
	1918											
Precipitation in inches	0.62	0.15	0.63	00	00	00	00	0.39	00	0.15	0.12	0.27
Departure from normal	.....	.....	+0.37	−0.07	00	00	−0.34	−0.49	−0.09	−0.09	−0.15	−0.14
	Comparative temperatures 1917											
Temperature	53.3	.....	59	65.7	69.2	81.2	88.4	86.6	84.2	74.4	60.3	56.6
Departure from normal	.....	.....	−1.5	+0.01	−1.5	+2.8	+2.4	+0.5	+4.9	+6.2	+1.5	+6.3
	1918											
Temperature	53.3	56.7	64.8	68.4	71.8	86.2	87.9	.....	83.7	73.6	58.6	51.6
Departure from normal	+1.6	+1.1	+4.3	+2.8	+1.2	+ 7	+1.9	.....	+3.7	+4.5	−0.3	+ .7

FIG. 4.—Progressive stages in mummification of date fruit, due to spot fungus; spots rusty brown at first, then dark chocolate to black, margined with lighter

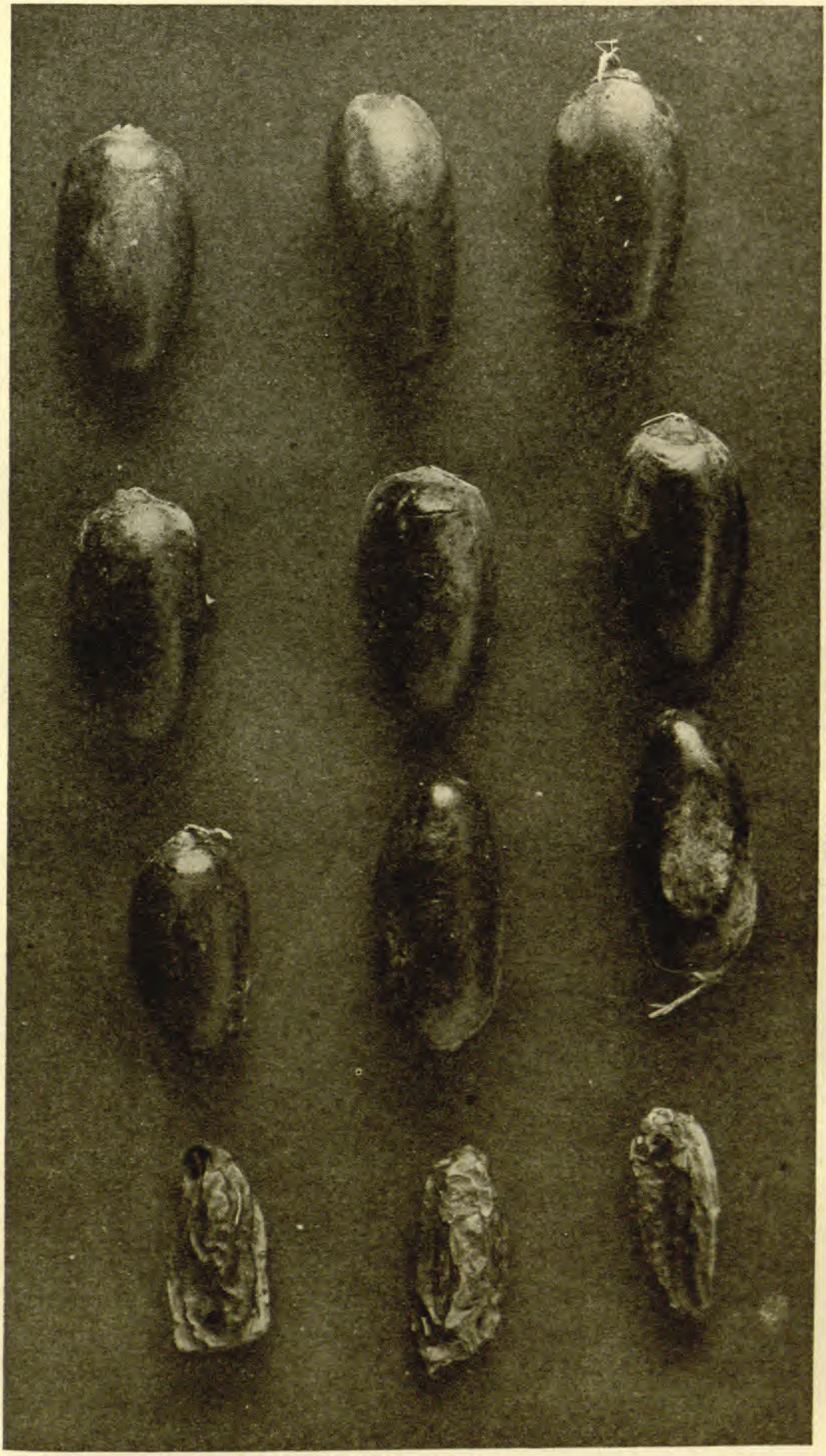

FIG. 4.—Progressive stages in mummification of date fruit, due to spot fungus; spots rusty brown at first, then dark chocolate to black, margined with lighter depressed centers.

dates for prunes. Infected spots in all three situations gave typical *Alternaria* spores similar to those found in pustules. Besides this fungus, two species of *Aspergillus* and one species of

FIG. 5.—Stages in rotting and drying out following attack of *Alternaria*, *Aspergillus*, and *Penicillium*, showing spots having a water-soaked appearance at first.

Penicillium usually appeared. The method of inoculation consisted in the removal of a block of infected tissue with a flamed scalpel after sterilization of the surface. The fungi appearing on the agar have all been isolated and grown in pure culture. On old

agar cultures numerous perithecium-like, carbonaceous structures have appeared, which are now under histological and physiological investigation. In no instance, thus far, have they produced spores on artificial media, nor do they appear on infected material that has been examined.

An important phase of the date rot problem is the difficulty with which stored fruits are kept. Since another investigator is working on methods of preserving date fruit, it will suffice to state that the same saprophytic molds that occur in infected petioles, rhachi, and fruits on the tree are likely later to ruin the packed crop.

CONCLUSIONS.—From the writer's preliminary observations and experiments, it appears probable that the primary cause of rot and mummification of the date fruit is the attack of *Alternaria*. This attack paves the way for the entrance of saprophytic *Aspergillus* and *Penicillium* species which bring on the disintegration of the pulp. Commonly all three fungi appear in cultures from the same infected spot. Attack of the spot fungus, unaccompanied by the saprophytic *Aspergillus* and *Penicillium*, results in mummification without the appearance of rot.

LITERATURE.—There appears to be no literature available on diseases of the date in this country, a statement borne out by communications from the Bureau of Plant Industry. Some European investigators have mentioned a species of *Sterigmatocystis* as the cause of the rot of date fruit in Africa, but this fungus has not yet appeared on any of the Arizona material examined. *Meliola* has also been mentioned in connection with the spot of the leaf of date palms, but it also is absent from cultures made in this laboratory. Further study is needed to determine the species of *Alternaria*, *Aspergillus*, and *Penicillium* isolated in cultures. This work, together with histological investigation and inoculation experiments, is already under way, and it is hoped that a further report may be ready soon.

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CURRENT LITERATURE

BOOK REVIEWS

Inbreeding and outbreeding

The latest of the series of "Monographs on Experimental Biology" is by EAST and JONES.[1] The book is noteworthy in bringing together in convenient form critical data on an important subject, which have heretofore been neither well organized nor readily accessible. Some will object to certain conclusions, but they enhance the value of the book as a monograph, and all assertions are supported by striking evidence.

Chapter i describes the nature of the problem, discusses its sociological, agricultural, and evolutionary significance, and emphasizes the necessity of proper experiments for its solution. Chapter ii outlines briefly the position of cross-fertilization in the evolution of reproduction in plants and animals. The conclusion is that those organisms which were able to cross with others, at least occasionally, have dominated the organic world. For DARWIN's negative proposition, "Nature abhors perpetual self-fertilization," a positive substitute is provided in, "Nature discovered a great advantage in an occasional cross-fertilization."

Chapter iii is a very condensed description of the rôle and behavior of chromosomes in plant and animal reproduction. Sex determination and sex linked inheritance are also included, although the reviewer doubts whether these are necessary. Chapter iv presents MENDEL's law, wisely using a 1:2:1 ratio as the type illustration, linkage, and the factor hypothesis, with particular emphasis on the inheritance of quantitative characters. The rôle of the factor as a mathematical concept, similar to the chemical atom, is ably presented. Chapter v discusses mathematically the effect of various types of inbreeding upon the heterozygosity of a population. Chapter vi presents the inbreeding experiments of CRAMPE, RITZEMA-BOS, WEISMANN, VON GUAITA, KING, ROMMEL, CASTLE, WHITNEY, and A. F. SHULL on animals, and of DARWIN, G. H. SHULL, and of the authors themselves on plants. The emphatic conclusion is that inbreeding is not injurious merely by reason of the consanguinity; apparent evil results are due merely to the isolation of certain recessives.

Chapter vii describes the experiments of KOLREUTER, KNIGHT, GARTNER, NAUDIN, MENDEL, FOCKE, DARWIN, EAST and HAYES, ROBERTS, COLLINS and KEMPTON, GERNERT, and SARGERET on hybrid vigor in plants.

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[1] EAST, E. M., and JONES, D. F., Inbreeding and outbreeding. 8vo. pp. 285. *figs. 46*. Philadelphia: J. B. Lippincott Co. 1919. $2.50.

The results involved increase in size (number as well as size of cells), weight, yield, early flowering, longevity, resistance to climate and disease, seed viability, and ease of vegetative propagation. Foreign pollen immediately produces larger endosperm (maize) than does own pollen in different seeds of the same ear.[2] No selective fertilization in favor of foreign pollen is associated with hybrid vigor.[3] Those characters which are quickest to be modified by external factors also show the greatest degree of hybrid vigor on crossing. On hybrid vigor in animals, which is less noticeable, the work of CASTLE is described, and that of some others briefly mentioned. Chapter viii sketches in historical sequence the theoretical mechanisms which have been provided to explain hybrid vigor, culminating with the junior author's explanation through "dominance of linked factors."[4] The faint possibility of fixing "hybrid" vigor is discussed. Chapter ix discusses the relation of the problem to sterility. Two distinct types of sterility occur: (1) inbreeding may isolate sterile strains in the same way that it isolates other characters; and (2) rather wide crosses may produce a sterile F_1 because through the degree of difference between the uniting germ plasms "the precise and complex machinery governing gametogenesis cannot do its work in the normal manner, and sterility results, although under the same conditions developmental (somatic) cell division goes on as usual."

Chapter x sketches the rôle of inbreeding and outbreeding in evolution. It contains a number of interesting speculations, among which the most striking are that hybrid vigor may be pictured as the efficient cause of the establishment of the sex habit itself, and of the rise of the sporophyte generation in plants. The authors differ from many geneticists in claiming that "bud variations occur much more frequently in heterozygotes than in homozygotes." Chapter xi outlines the value of inbreeding and outbreeding in plant and animal improvements. "There must be cross-breeding to furnish a variety of character combinations from which to select; there must be inbreeding to isolate the combinations desired." The practical utilization of hybrid vigor is pictured also, a fairly common practice in live stock breeding, a rare one in plants other than maize, for which the authors commend a double crossing system.

The two concluding chapters present applications to the human race. Although avowedly less exact than the earlier chapters, these are in a sense the most interesting of all, as may appear in the following summarizing quotations.

"Owing to the existence of serious recessive traits there is objection to indiscriminate, irrational, intensive inbreeding in man; yet inbreeding is the surest means of establishing families which as a whole are of high value to

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[2] JONES, D. F., Bearing of heterosis on double fertilization. BOT. GAZ. **65**:324–333. *figs.* 3. 1918.

[3] BOT. GAZ. **68**:150. 1919.

[4] BOT. GAZ. **66**:70. 1918.

the community. On the other hand, owing to the complex nature of the mental traits of the highest type, the brightest examples of inherent ability have come and will come from chance mating in the general population, the common people so-called, because of the variability there existent. There can be no permanent aristocracy of brains, because families, no matter how inbred, will remain variable while in existence and will persist but a comparatively short time as close-bred strains. But he is a trifler with little thought of his duty to the state or to himself, who, having ability as a personal endowment, does not scan with care the genealogical record of the family into which he enters."

"The hybridization of extremes is undesirable because of the improbability of regaining the merits of the originals, yet hybridization of somewhat nearly related races is almost prerequisite to rapid progress, for from such hybridization comes that moderate amount of variability which presents the possibility of the superindividual, the genius. . . . . Further, there must be periods of more or less inbreeding following racial mixtures, if there is to be any high probability of isolating desirable extremes. A third essential in the production of racial stamina is that the ingredients in the melting pot be sound at the beginning, for one does not improve the amalgam by putting in dross."

One of the most valuable features of the book is the admirable bibliography of 225 titles.—M. C. COULTER.

NOTES FOR STUDENTS

Temperature and the cobalt chloride method of measuring transpiration.—In the improvement by LIVINGSTON and SHREVE[5] of STAHL'S cobalt method of measuring transpiration, one question has remained unanswered, namely, Is it sufficiently accurate to regard the temperature of the slip as it lies on the leaf as being the same as that of the surrounding air? SHREVE[6] has answered this question by making use of a thermo-electrical method for measuring leaf temperatures. This method differs from previous ones in the avoidance of the wounding of the leaf and the resulting temperature complications. Using this method, SHREVE has demonstrated that both in the determination of the index of transpiring power by cobalt slips, and in the standardizing of the slips themselves over a porous evaporating surface, no error results from using the temperature of the air surrounding the apparatus instead of the temperature of the slips themselves.—S. V. EATON.

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[5] LIVINGSTON, B. E., The resistance offered by leaves to transpirational water loss. Plant World **16**:1–35. 1913.

LIVINGSTON, B. E., and SHREVE, E. B., Improvements in the method for determining the transpiring power of plant surfaces by hygrometric paper. Plant World **19**:287–309. 1916.

[6] SHREVE, E. B., A thermo-electrical method for the determination of leaf temperature. Plant World **22**:100–104. *figs. 2*. 1919.

———, The rôle of temperature in the determination of transpiring power of leaves by hygrometric paper. Plant World **22**:172–180. *fig. 1*. 1919.

Anatomy of strand plants.—The eastern shore of Madagascar, characterized by uniformly high temperature, constant winds, and considerable rainfall, has as its principal strand communities two associations characterized respectively by *Ipomea Pes-caprae* and *Barringtonia*. The plants of the former have been examined by DENIS,[7] who finds them, thus exposed to conditions of high transpiration, induced by great insolation and rapid air movement, almost without any special development of epidermal protection, but possessing varying degrees of fleshiness with water-storing tissue rather well developed. One group shows isolateral fleshy structure with the water tissue centrally placed, another possesses bifacial leaves, less fleshiness, and peripheral water tissue. These structural tendencies toward fleshiness are related by the author to the saline character of the beach; the development of water-storing tissue and the early lignification of the roots to the high rate of transpiration; and the abundance of palisade tissue to brilliancy of both the direct and the reflected sunlight. The details of structure are given in the text and in the drawings of leaf sections.—GEO. D. FULLER.

Snow and timber line.—From studies made in the Pyrenees, BOUGET[8] has reached conclusions regarding the influence of snow upon alpine and subalpine vegetation not unlike those of SHAW[9] from a study of the Selkirks more than a decade ago. In the higher altitudes the duration of the snow is related to the local topographic relief, and its persistence during the growing season profoundly influences the character of the vegetation. In depressions it collects during the winter and remaining late in the season gives rise to a rather mesophytic herbaceous community consisting of a mixture of lowland and alpine forms. In contrast, the relative absence of snow upon the ridges and at the same altitude produces a xerophytic vegetation in which trees and woody plants are conspicuous. Thus the upper limit of trees or timber line is much higher upon ridges than along depressions.—GEO. D. FULLER.

Action of enzymes on cellulose.—PRINGSHEIM and MAGNUS-VON MERKATZ[10] point out that dextrines from both starch and glycogen are split to maltose by diastase. They raise the question whether diastase has a similar effect on cellulose dextrine. By using MADSEN's acetylization method they gained cellulose dextrine from cotton that was soluble in water and

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[7] DENIS, MARCEL, Recherches anatomiques sur quelques plantes littorales de Madagascar. Rev. Gén. Botanique **31**:33–52, 115–120, 129–142. *pl. 1. figs. 12.* 1919.

[8] BOUGET, J., De l'influence des neiges sur la répartition des différents végétaux à mène altitude dans les zones élevées des Pyrénées. Rev. Gen. Bot. **30**:305–320. 1918.

[9] SHAW, C. H., The causes of timber line on mountains; the rôle of snow. Plant World **12**:169–181. *figs. 4.* 1909.

[10] PRINGSHEIM, H., and MAGNUS-VON MERKATZ, A., Fermentversuche an Zellulose abbauprodukten. Hoppe-Seyler Zeit. Physiol. Chem. **105**:173–178. 1919.

gave no osazone reaction. The dextrine thus obtained is strongly reducing to Fehling's solution, and is considered by the authors as the end dextrine of cellulose. Diastase will not split cellulose dextrine. They also derived zellobiose by the MADSEN method. The contents of the first stomach of cattle, the intestine, and the pancreas bore no enzyme that would split zellobiose. They conclude that the splitting of this substance in the alimentary canal of the cattle must be due to cellulose bacteria.—WM. CROCKER.

Ecological diversity and generic coefficients.—The principle first enunciated by JACCARD, and noted in this journal,[11] that the ratio between species and genera, or the generic coefficients, varies inversely with the diversity of the habitat conditions, has received additional support from the investigations of DUFRENOY[12] upon the distribution of parasitic fungi in different habitats. Diversity of ecological conditions was found at altitudes of 1100 m. to 2000 m. in the valley of Barèges, where the generic coefficient for rusts was 20 per cent and for all fungi 40 per cent. Contrasted with this were the uniform conditions in a wheat field showing generic coefficients for its fungi of 70 per cent, and upon sand dunes with coefficients ranging from 90 to 100 per cent.—GEO. D. FULLER.

Lignins.—PRINGSHEIM and MAGNUS,[13] in a study of lignins, have obtained some interesting results. When wood or straw is treated with sodium hydrate in the cold, all the acetic acid liberated is derived from the lignins of these materials. When these materials are boiled with sodium hydrate, either under pressure or otherwise, most of the acetic acid formed is derived from the lignins; but a small part is derived from the cellulose and none from the pentoses. The lignin of the white beech yields about 37.85 per cent of its weight of acetic acid, and the lignin of conifer wood about 19.48 per cent. —WM. CROCKER.

Dioecism in Thalictrum.—SCHAFFNER[14] has studied *Thalictrum dasycarpum* in reference to intergrades between the monoecious and dioecious condition. It seems to be a peculiarly favorable form for this purpose, and almost every conceivable intermediate in the expression of "maleness" and "femaleness" was found. The author rightly calls attention to the fact that the physiological and ecological factors concerned in these various expressions must be taken into consideration for an understanding of the evolutionary changes leading from the bisporangiate to the monosporangiate condition.—J. M. C.

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[11] BOT. GAZ. **57**:540. 1914.

[12] DUFRENOY, J., Diversité écologique et coefficients génériques. Bull. Trim. Soc. Mycol. Fr. **35**:27–46. 1919.

[13] PRINGSHEIM, H., and MAGNUS, H., Über den Acetylgehalt des Lignins. Hoppe-Seyler Zeit. Physiol. Chem. **105**:179–186. 1919.

[14] SCHAFFNER, J. H., Dioeciousness in *Thalictrum dasycarpum*. Ohio Jour. Sci. **20**:25–34. 1919.

Fungi of North Carolina.—COKER[15] has published a systematic account of certain genera of fungi occurring in North Carolina, illustrated by remarkably fine plates. *Craterellus* is represented by 7 species and *Cantharellus* by 12. *Eomycenella*, *Tragia*, and *Nyctalis* are each represented by a single species. A very complete key to the genera of gill fungi (55 genera) is also included. —J. M. C.

New genus of Pyrenomycetes.—Fitzpatrick[16] has described a new genus (*Rostronitschkia*) of Pyrenomycetes from Porto Rico and Jamaica, parasitic on the leaves of *Gesneria albiflora*. The species has been known for some time, and described by REHM (in litt.) as a new species of *Nitschkia* (*N. nervinicola*). FITZPATRICK has concluded that it is distinct enough from that genus to be entitled to separate generic rank.—J. M. C.

Mutation.—Costantin[17] has published a full discussion of the status of the theory of mutation, weighing the evidence for and against it, as presented in the extensive literature of the subject. His conclusion is that, in spite of the violent assaults against it, "la theorie de la mutation reste debout." The details of the discussion will be well worth consulting by those interested in this controversy.—J. M. C.

Suspensor of Capsella.—SOUÈGES[18] has made a remarkably detailed study of the early embryogeny of *Capsella*, especially in reference to the suspensor. The numerous details cannot be repeated in a brief review, but they should become familar to morphologists. That so much could be obtained from a form so commonly studied is remarkable.—J. M. C.

Tropical American Eupatoriums.—ROBINSON,[19] in continuing his studies on the South American species of *Eupatorium*, has paid particular attention to those of Peru and Bolivia. In the present contribution 37 new species are described, and a review of the Peruvian material recognizes 82 species. —J. M. C.

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[15] COKER, W. C., *Craterellus*, *Cantharellus*, and related genera in North Carolina; with key to the genera of gill fungi. Jour. Elisha Mitchell Sci. Soc. **35**:24–48. *pls. 17*. 1919.

[16] FITZPATRICK, H. M., *Rostronitschkia*, a new genus of Pyrenomycetes. Mycologia **11**:163–167. *pl. 11*. 1919.

[17] COSTANTIN, J., La mutation, état actuel de la question. Ann. Sci. Nat. Botanique X **1**:iii–xxxii. 1919.

[18] SOUÈGES, R., Les premières divisions de l'oeuf et les différenciations du suspenseur chez le *Capsella Bursa-pastoris* Moench. Ann. Sci. Nat. Botanique X **1**:1–28. *figs. 69*. 1919.

[19] ROBINSON, B. L., I. On tropical American Compositae, chiefly Eupatorieae. II. A recension of the Eupatoriums of Peru. Proc. Amer. Acad. **55**:1–88. 1919.

Fumaria and Rupicapnos.—PUGSLEY[20] has published a very detailed revision of *Fumaria* and also of *Rupicapnos* whose species were formerly included in *Fumaria*. In *Fumaria* 46 species are recognized, 6 of which are new; in *Rupicapnos* 20 species are recognized, 7 of which are new.—J. M. C.

New genus of Rubiaceae.—REHDER[21] has described a new genus (*Tetraplasia*) of Rubiaceae from the Liukiu Islands, an evergreen shrub endemic to the islands. It is thought to belong to the Vanguerieae, and is most closely related to *Plectronia* and *Vangueria*.—J. M. C.

New conifers.—WILSON[22] has discussed four new Korean conifers, one, a *Thuja*, having been described in 1919 by NAKAI. The others described by WILSON are a new species and a new form of *Abies*, and a new form of *Larix*. —J. M. C.

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[20] PUGSLEY, H. W., A revision of *Fumaria* and *Rupicapnos*. Jour. Linn. Soc. Bot. **44**:233–355. *pls. 9–16*. 1919.

[21] REHDER, ALFRED, *Tetraplasia*, a new genus of Rubiaceae. Jour. Arnold Arboretum **1**:190–191. 1920.

[22] WILSON, E. H., Four new conifers from Korea. Jour. Arnold Arboretum **1**:186–190. 1920.

GENERAL INDEX

Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold face** type; synonyms in *italic*.

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