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CONTENTS

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A BIOLOGICAL AND STATISTICAL ANALYSIS OF THE VEGETATION OF A TYPICAL WILD HAY MEADOW.

A. B. STOUT

INTRODUCTION.

Natural low lying meadows are important sources of wild hay supply over a considerable portion of the northern states. This is especially true of the region about the city of Madison, Wisconsin, where many acres of "marsh hay," "wild hay," or "slough hay" lands are cut yearly. The vegetation of such habitats has been recognized by all students of plant geography and ecology as a more or less definitely characterized formation consisting largely of sedges and grasses. Little attention has been given to the detailed field study of this class of vegetation. The improvement and utilization of marsh lands is however receiving more and more attention and this vegetation is destined to become an object of increased interest both from economic and scientific points of view.

The hay cut from these marsh meadows varies in value in the local markets from four or five dollars to as high as eight or nine dollars per ton. The basis of distinction in value is doubtless to be found in the varying ratio of grasses to sedges and the degree of coarseness of the latter. The grasses are regarded as having higher feeding value although little distinction is made between them and such grass-like sedges as *Carex Sartwellii* and *Carex stricta.*

The earlier writers on plant geography noted that marsh meadows are typically northern in their geographical distribu-

tion. In regard to this point Schouw (1) (1823), who mapped the earth's surface into kingdoms and provinces on the basis of families peculiar to each region, made a "Province of Carex" which he located in the region of the Arctic Circle.

Meyen (2) (1836) regarded Gramineous plants as an important physiognomic class and noted that the low meadow forming grasses are characteristic of colder regions while grasses of the tropics are chiefly of larger or gigantic size. "Meadows," he says, "are an ornament of northern regions."

These general statements of Schouw and Meyen in regard to sod forming grasses and sedges, are accepted by Grisebach (3) (1872) who makes the additional observation that one tenth of all the vascular plants of the Arctic regions belong to the genus Carex. He notes that the Carices predominate on the more poorly drained areas while sod forming grasses prevail on the better drained areas. In his discussion of the forest areas of both the Eastern and the Western Continents he states that there is also present in the same latitude a series of meadow formations depending on the drainage conditions just as there is in the Arctic regions.

Coming to the more recent students of plant geography, Drude (7) (1890) distinguishes more closely between meadows, as composed largely of grasses, and meadow moors composed largely of sedges, the latter with a peaty substratum having a high water content. His classification of these low moist sod formations is as follows:

A, Dormant during winter; during the warm seasons growing and green, composed of low growing shrubs and herbs, and moss; trees are absent, and turf is compact—

a Principally grasses with short sward: Meadows.

b Principally of sedges, reed grasses with short sward growing on peaty marshy soil: Grass moors. Meadow moors.

In this distinction the emphasis is placed on the character of the vegetation. Drude again emphasizes the fact that of the three thousand species of Cyperaceae relatively few are found in the tropics, the genus Carex being characteristically circumpolar in its distribution. He also describes *Carex stricta* as an

important factor in the natural filling in of ponds leading to the development of meadow lands.

Drude's meadow moor is plainly the equivalent of our marsh meadows. If he had distinguished further the grass moor and sedge moor according to the prevalence of such grasses as *Calamagrostis canadensis* he would have characterized these formations quite adequately, judged from the standpoint of our Wisconsin conditions.

The presence of this type of vegetation in Wisconsin was recognized by Chamberlin (5) (1877) and his co-laborers. In their discussion of the flora of Eastern Wisconsin they recognized a "Grass and Sedge Group" occupying open meadow and marshes and they note that grasses predominate on marshes more easily improved and that sedges occupy marshes more difficult to improve. They find that this form of vegetation grades imperceptibly into the upland prairies. A sharp distinction is made by them between the "Grass and Sedge Group" and the "Heath Group" though both occupy marsh habitats. The map of the vegtation of Wisconsin which accompanies their discussion shows that the grass and sedge marshes are confined to the west central portions of the state and are intermingled with the prairies in the belt of the greatest development of the oaks. To the immediate north and east the "Heath Group" reaches its greatest development in the marshes. In the dense pine and maple belt the tamarack is shown to be characteristic of the undrained regions while still further north this species is largely replaced by white cedar and spruce.

MacMillan (9) (1892) whose work on the flora of Minnesota stands quite alone in its comprehensiveness so far as any of our western states are concerned, describes the type of vegetation with which we are concerned as a "swamp moor or wet meadow" formation composed mostly of "sedges, grasses, and rushes, but with a strong intermixture of other plants including such varieties as the shield ferns, marsh marigolds, the Parnassias, some gentians, buck beans, orchids, willow herbs and parsleys." Mingled with these are often shrubs such as dogwoods, willows, buckthorns and meadow sweets, and also a considerable moss vegetation.

For the Minnesota River Basin he lists two hundred and sixty-four marsh and swamp inhabiting plants but does not specify in this list the "swamp moor" element. Of this list one hundred and forty-five are monocots with seventeen grasses and forty-six species of Carex. From the available literature MacMillan shows that these swamp plants are mostly northern and eastern in their distribution and that they constitute twenty-two and one-half per cent of all the species of the Minnesota Valley. He does not, however, give any data as to the relative abundance of these classes of plants.

Pound and Clements (12) (1900) in their map of the so-called "Prairie Province" show that the eastern line of blending of this with the "Forest Province" passes diagonally across Wisconsin in the region of the greatest development of marsh meadows as shown on Chamberlin's map of the vegetation of Wisconsin. In Nebraska they distinguish a "wet meadow formation intermediate between marshes and meadows proper," and subdivide it into three types, as follows: (1) The rush meadow type, (2) The fern meadow type, and (3) the sedge meadow type. The latter is composed largely of *Carex stricta, Carex striata* and *Carex lanuginosa.* According to their observations accessory species are almost lacking. Extensive marsh meadows of this type exist in Nebraska only along the main streams near the Missouri River. A characteristic swamp meadow formation is described by Beck (13) (1901) as present in the Upper Balkan peninsula. The "Sumpfwiesen" as he calls it, is composed largely of sedges and reaches its best development in the broad lower valleys. He lists one hundred and twenty-two species of plants characteristic of such meadows. Of these forty-five are monocots, fourteen are grasses, and nineteen are sedges of which twelve are Carex species. In his list are such species as the following: *Phragmites communis, Molinia coerulea, Poa palustris, Scirpus lacustris, Carex vulpina,* and *Carex riparia.* Statistics as to relative abundance are lacking hence it is difficult to compare this type with our wild hay meadows.

Ganong (15) (1903) finds among the fresh water marshes

of the region about the Bay of Fundy a "wet meadow" formation characterized by two types of vegetation. In one *Spartina cynosuroides* Willd. is dominant with *Cicuta maculata, Carex maritima, Calamagrostis canadensis,* and *Scirpus atrovirens* as secondary species. He also finds a floating bog association or "Caricetum" with *Carex filiformis, Eriophorum vaginatum, Carex stricta decora,* and *Carex Magellanica* as principal species.. Both of these have species in common with our marsh meadows but all being more nearly a bog formation on the flood plain of a tidal river.

Lewis (17) (1904) describes, under the term "Grass Heath" a formation of the Pennine moors of England which although differing somewhat has many points in common with our marsh meadows. It is developed on glacial drift overlaid by peat with a high water content. The grass *Molinia varia* is dominant with such secondary species as *Deschampsia flexuosa, Carex flava, Carex echinata, Carex Goodenovii,* and *Juncus conglomeratus.* He distinguishes these sharply from the Heath (Heide or Zwergstrauchheiden of Warming). He states that these grass heaths stretch for miles on gently sloping poorly drained ground, and that although they occur at all altitudes they are best developed at one thousand feet altitude where they form intermediate zones between the heather moors higher up and the cultivated lands of lower altitudes.

Smith (18) (1904) makes a sharp distinction between typical bog formations and "marshes" in Scotland. The latter he considered as corresponding to the typical meadow moor of Warming. He found that in Scotland their vegetation was composed of the taller sedges, grasses and rushes, and that they were best developed on the "landward side of the reed swamps of lowland lochs and rivers."

In the various types of vegetation present in the extensive flat-moors of Austria, Bailer and Wilk (22) (1907) have described associations which are quite similar to the wild hay meadows of Wisconsin. They term such an association a "Magno Caricetum" and describe it as composed chiefly of *Carex stricta, Carex filiformis, Carex acuta, Carex paludosa,*

Carex vesicaria, and *Carex riparia,* with *Equisetum limosum, Equisetum palustris,* Hypnum species, and *Cladium maricus* as secondary species, and as less frequent *Menyanthes trifoliata* and *Heleocharis palustris.* They also note that "on drier places this association is quickly replaced by a Molinietum" or a more purely grass association.

Feilburg (8) (1890) has studied the influence of the depth of the ground water on the vegetation of marshy sand plains in Jutland. He claims that, the chemical and physical composition of the soil being uniform, "when the ground water in summer stands at a depth of three inches Juncus and meadow moor vegetation prevail; at six inches mosses and Cyperaceae still occur but grasses begin to appear; at nine inches these taller become dominant; at twelve inches normal grass growth occurs in ordinary summers; at from eighteen to twenty-four inches cereals thrive in cold moist summers, at from thirty to forty inches the soil is unsuited for cereals and xecophytes predominate." According to Feilburg the depth of the water table is here the sole factor determining the natural vegetation that appears and the crops that can be grown.

As to the physical characteristics, soil composition and distribution, Whitson and Jones (23) (1907) distinguish two types of marsh lands in Wisconsin. The one most abundant in Northern Wisconsin is in the sandstone and granite regions, as a rule, and is the typical bog formation with sphagnum, tamarack, and spruce. The soil is strongly acid and is deficient in available potash and phosphoric acid. The second type is the marsh meadow or meadow moor best developed on the lime stone area of the eastern and south eastern part of the state. Here the peat shows little or no acidity, is deficient in potash but less so in phosphoric acid. To this latter type belong the wild hay marshes of this portion of the state

The region about the city of Madison is especially favorable for the study of marsh formations and I have undertaken to analyze the flora of a typical formation of this class by statistical methods with the aim of obtaining the numerical relations, and the relative importance of the various species as they are grouped in such an association.

The area studied is chiefly included in the property of the Dane County Fair Association and is one of the many wild hay meadows in the vicinity of Madison. In its general outline this marsh meadow is almost circular and it is nearly surrounded by low irregular morainic knolls. The east edge has been cut by the road beds of the Chicago, Milwaukee and St. Paul Railway, and the western side by that of the Chicago and Northwestern Railway. At the south edge a race track has been built out into the marsh. A few large ditches have been dug mainly for the drainage of the race course. Formerly a shallow, sluggish water course known as Murphy Creek meandered through the center serving as an overflow outlet from Lake Wingra but its function is now taken by a canal recently cut between Lakes Wingra and Mendota. A broad strip of marsh extending through the center from north to south is at present little affected by these changes and it is this region that I have especially studied.

The area is almost perfectly flat as is shown in the photographs here reproduced. The elevation is but a few feet above the level of the lake which lies at its nearest point but a few rods from the eastern border of the marsh. During the spring and after heavy summer rains water floods much of the area to a depth of several inches.

Ten soil borings made at points along the transect showed that the surface layer of humus muck and well rotted peat is from one to four feet in depth. There is a shallow layer at the south edge where for a distance of one hundred and twenty-five feet it is underlaid by sand, fine gravel, and red clay at a depth of from a few inches to one and one-half feet. The greater portion of the central part has about four feet of peaty material underlaid by a fine grained blue clay. A broad belt at the north is underlaid by white sand. The water table in these holes, bored during the autumn of 1908 at an unusually dry time, varied from a few inches beneath the surface in the central portions to three feet or more in the portions underlaid by sand.

In common with the numerous marshes of this part of Wis-

consin, as pointed out by Chamberlin (5) (1877), this marsh meadow has probably been formed chiefly by the accumulation of vegetable material in a rather shallow expanse of lake area. The region is one of rather imperfect drainage due to the glacial deposits associated with the terminal moraines. The clay and the sand substrata undoubtedly formed the bottom of the former body of water and the accumulation of plant substance accounts for the rather thin layer of peat and muck which now holds the rainfall like a sponge. Erosion of the surrounding knolls has resulted in washing more or less of the upland soils on to the borders of the area.

The entire area is well clothed with a dense growth of grasses and sedges which form a well knit turf of sufficient firmness over much of the area to hold up teams in the work of hay making, at least during a dry mid-summer. If cut at all the hay is usually made during August although the work is often delayed until the ground is frozen in which case the product is more often used as bedding or for packing ice, etc.

Method of Study

The frequence and the abundance of the various species of plants on the area were studied by countings of the plant population on a strip four inches wide extending from edge to edge through the center of the marsh in a north and south direction. It was planned to extend this belt-transect in a straight line across the entire marsh but when the part north of the canal was reached it was found that cattle had eaten out portions making a slight deviation from a continuous straight line necessary. This belt-transect fairly represents the conditions of the whole area and its population was carefully studied as to both the occurrence and abundance of all the species concerned.

The enumeration was planned to give complete data throughout the transect in regard to—(1) the species present, (2) the numerical abundance of each species, (3) the numerical frequence, (4) the grouping of the species, and (5) the relative weight of the dominant species in the hay produced.

In making the counts a ten foot measuring rod was pushed

through the grass close to the surface of the ground. Then the vegetation at one side was trampled down as shown in the photographs of plates XX and XXI. A comb or rake like device was then used to determine definite areas of uniform size. This comb was one foot long and had four pointed teeth each four inches apart measuring from tip to tip. It could be pushed into the vegetation at any point along the rod and when thus in place it enclosed on three sides the vegetation growing on three four inch square areas, or quadrats, which were the unit areas on which the different species present were noted and their number determined by count. With note book previously ruled and numbered the data were recorded for each quadrat of every other foot, thus data were obtained in successive order representing rather completely all the conditions on the area. A stake was placed at the end of each ten foot segment. These stakes were numbered and left standing throughout the season so that continuous observations could be taken concerning seasonal changes in growth. Plants undetermined at the time of the census because of immaturity were staked and labelled to await the development of the floral parts. It was impossible to determine the individual plants of the various grasses and sedges. These types have a more or less highly developed underground stem system. In *Carex stricta, Carex riparia, Carex aquatilis* and *Carex filiformis,* especially, the underground stems branch out in all directions from the individual stools. (See Plates XXI and XXII). This method of propagation gives a rather widely spread plant if we consider that all the clumps of culms having direct connection with each other constitute a plant. The result gives an intermixing not only of plants of different species but of the same species. For the grasses and sedges it was hence deemed best to count the culms.

The species of Eleocharis were not counted. Although widely distributed in the marsh and exceedingly abundant in point of number of culms, they were everywhere weak and slender and overshadowed by more vigorous species. The abundance of the Eleocharis species is given in the relative terms, absent, sparse, medium dense, and dense.

The entire length of the belt transect counted was two thousand three hundred feet. It was four inches wide and all plants on every other foot were counted with the exception of the Eleocharis species. Thus one thousand one hundred and fifty records were made on areas twelve by four inches, that is, the plants on three thousand four hundred fifty areas of four inches square were counted.

A few species were noted growing near the belt transect but not actually included in it. With the exception of a few species these were plants not dominant on any portion of the marsh. To give completeness to the list these species are included and are indicated by a star.

The nomenclature is that of Gray's Manual (seventh edition). All species not identified with certainty were sent to the United States National Herbarium where they were determined by Mr. C. F. Wheeler. The mosses included in the list were determined by Mr. A. J. Grout.

List of Species Growing on The Four Inch Strip.

Musci
- Calliergon Schreberi, Wild.
- Campylium chrysophyllum Bryhn.
- Drepanocladus uncinatus (Hedw.) Warnst.
- Bryum (?).

Polypodiaceae
- Aspidium Thelypteris (L) Sw.

Equisetaceae
- Equisetum arvense L.
- Equisetum fluviatile L.

Typhaceae
- Typha latifolia L.

Sparganiaceae
- Sparganium eurycarpum Engelm.

Alismaceae
- Sagittaria latifolia Willd.

Gramineae
- Andropogon furcatus Muhl.
- * Phleum pratense L.
- Agrostis alba L.
- Calamagrostis canadensis (Michx) Beauv.
- Calamagrostis neglecta (Ehrh.) G. M. and S.
- Sphenopholis pallens (Spreng.) Scribn.
- Spartina Michauxiana Hitchc.

* Phragmites communis Trin.
Poa pratensis L.
Glyceria nervata (Willd.) Trin.
Bromus ciliatus L.
Hordeum jubatum L.

Cyperaceae

Eleocharis palustris (L.) R. and S.
Eleocharis acicularis (L.) R. and S.
Scirpus validus Vahl.
* Scirpus atrovirens Muhl.
Eriophorum angustifolium Roth.
* Carex straminea Willd.
Carex Bebbii Olney.
Carex sterilis Willd.
* Carex vulpinoidea Michx.
Carex diandra, var. ramosa (Boott.) Fernald,
* Carex stipata Muhl.
Carex Sartwellii Dewey.
Carex aquatilis Wahlenb.
Carex stricta Lam.
Carex tetanica Schkuhr.
Carex polygama Schkuhr.
Carex filiformis L.
Carex lanuginosa Michx.
Carex riparia W. Curtis.
* Carex comosa Boott.
* Carex hystericina Muhl.

Juncaceae

Juncus Dudleyi Wiegand.

Liliaceae

* Lilium canadense L.

Amaryllidaceae

Hypoxis hirsuta (L.) Coville.

Iridaceae

Iris versicolor L.

Orchidaceae

* Calopogon pulchellus (Sw.) R. Br.

Salicaceae

Salix discolor Muhl.
Salix candida Flügge.
* Salix amygdaloides Anders.

Santalaceae

Comandra umbellata (L.) Nutt.

Polygonaceae

* Rumex altissimus Wood.
* Rumex crispus L.
Polygonum amphibium var. Hartwrightii Gray.
* Polygonum pennsylvanicum L.

Ranunculaceae

* Ranunculus abortivus L.
* Ranunculus pennsylvanicus L. f.

Thalictrum dasycarpum F. and L.
Caltha palustris L.

Saxifragaceae
* Saxifraga pennsylvanica L.
Parnassia caroliniana Michx.

Rosaceae
* Spiraea salicifolia L.

Leguminosae
Trifolium pratense L.
Trifolium hybridum
Lathyrus palustris L.

Balsaminaceae
Impatiens biflora Walt.

Violaceae
Viola cucullata Ait.
Viola blanda Willd.

Lythraceae
Lythrum alatum Pursh.

Onagraceae
* Epilobium densum Raf.

Umbelliferae
* Zizia aurea (L.) Koch.
Sium cicutaefolium Schrank
Cicuta maculata L.

Primulaceae
Lysimachia thyrsiflora L.

Gentianaceae
* Gentiana crinita Froel.
* Gentiana Andrewsii Griseb.

Asclepiadaceae
Asclepias incarnata L.

Polemoniaceae
Phlox pilosa L.

Verbenaceae
* Verbena hastata L.

Labiatae
Scutellaria galericulata L.
Pycnanthemum virginianum (L.) D. and J.
Lycopus uniflorus Michx.
Lycopus americanus Muhl.
Mentha arvensis var. canadensis (L.) Briquet.

Scrophulariaceae
Chelone glabra. L.
* Mimulus ringens L.
* Gerardia purpurea L.
* Pedicularis lanceolata Michx.

Plantaginaceae
Plantago major L.

Rubiaceae
* Galium boreale L.
Galium trifidum L.

Campanulaceae
Campanula aparinoides Pursh.
Lobeliaceae
Lobelia siphilitica L.
Compositae
Eupatorium purpureum L.
Eupatorium perfoliatum L.
Solidago uliginosa Nutt.
Solidago neglecta T. and G.
* Solidago canadensis L.
* Solidago Ridellii Frank.
Aster puniceus L.
* Aster prenanthoides Muhl.
* Aster paniculatus Lam.
Erigeron philadelphicus L.
Helianthus grosseserratus Martens.
Bidens frondosa L.
* Bidens connata Muhl.
* Bidens trichosperma (Michx) Britton.
Cirsium muticum Michx.

A summary of the families, genera, and species is given in the following table:

TABLE I.

	Families.	Genera.	Species.
Musci		4	4
Pteridophyta	2	2	3
Monocots	10	23	42
Dicots	24	42	61
		71	110

ABUNDANCE OF THE VARIOUS SPECIES

The above summary shows that in point of number of species the dicots exceed the monocots but it is self evident that a mere list or summary of species does not show the actual occupancy of the area as it does not consider the numerical abundance of a species or the comparative number of individuals.

As a matter of convenience the data obtained by counting as previously described are here grouped in Table 2. In this list the species are arranged in the order of their numerical abundance. The strip is divided on the basis of its physical character and its plant population into five associations as follows: (1) Lycopus Caricetum, (2) Caricetum, (3) Calamagrostis Caricetum, (4) Lycopus Caricetum, (5) Caricetum.

TABLE 2.

	Lycopus Caricetum 1 to 500 ft.	Caricetum 500 to 750 ft.	Calamagrostis Caricetum 750 to 1,500 ft.	Lycopus Caricetum 1,500 to 2,250 ft.	Caricetum 2,250 to 2,350 ft.	Total.
Carex stricta	5,853	3,636	4,165	5,987	1,486	21,127
Calamagrostis canadensis	1,481	1,382	4,887	1,964	51	9,765
Carex sterilis	703	65	3	2,549	.....	3,320
Carex Sartwellii	43	28	2,934	344	.....	3,349
Lycopus uniflorus	522	253	.....	1,570	8	2,353
Carex aquatilis	.....	6	2,020	7	.....	2.033
Carex diandra var. ramosa	1,076	.....	67	593	204	1,940
Glyceria nervata	809	.....	.....	656	105	1,570
Carex filiformis	.....	12	1,229	122	.....	1,363
Agrostis alba	597	.....	.....	5	.....	602
Carex Bebbii	470	47	.....	3	27	547
Poa pratensis	326	.....	.....	.....	.....	326
Scutellaria galericulata	13	3	.....	232	37	285
Andropogan furcatus	58	.....	.....	213	.....	271
Viola cucullata	62	.....	.....	151	31	244
Aspidium Thelypteris	8	.....	12	202	.....	222
Carex riparia	.....	5	94	51	.....	150
Lycopus americanus	27	3	.....	118	.....	148
Sagittaria latifolia	1	4	92	1	.....	98
Sphenopholis pallens	.....	.....	25	61	.....	86
Campanula aparinoides	26	16	.....	40	.....	82
Parnassia caroliniana	2	.....	.....	79	.....	81
Viola blanda	.....	.....	.....	70	.....	70
Lathyrus palustris	5	.....	12	43	.....	60
Juncus Dudleyii	56	.....	.....	.....	.....	56
Galium trifidum	.....	.....	.....	53	.....	53
Thalictrum dasycarpum	19	.....	.....	32	.....	51
Mentha arvensis var	5	.....	.....	43	.....	51
Bidens frondosa	.....	.....	.....	49	.....	49
Lythrum alatum	.....	.....	.....	49	.....	49
Caltha palustris	10	.....	.....	37	.....	47
Aster puniceus	.....	.....	.....	45	.....	45
Sparganium eurycarpum	.....	.....	15	29	.....	44
Polygonium amphibium var	.....	.....	22	18	.....	40
Solidago uliginosa	.....	.....	.....	39	.....	39
Carex tetanica	16	.....	.....	22	.....	38
Salix discolor	.....	.....	.....	37	.....	37
Comandra umbellata	37	.....	.....	.....	.....	37
Calamagrostis neglecta	36	.....	.....	.....	.....	36
Equisetum arvense	29	.....	.....	.....	.....	29
Helianthus grosseserratus	4	.....	.....	23	.....	27
Scirpus validus	.....	.....	3	23	.....	26
Poa triflora	23	.....	.....	.....	.....	23
Impatiens biflora	22	.....	.....	.....	.....	22
Solidago neglecta	.....	.....	.....	18	.....	18
Carex polygama	.....	.....	.....	16	.....	16
Chelome glabra	.....	.....	1	12	.....	13
Phlox pilosa	.....	.....	.....	12	.....	12
Hypoxis hirsuta	.....	.....	.....	11	.....	11
Typha latifolia	.....	.....	7	4	.....	11
Equisetum fluviatile	.....	.....	.....	10	.....	10
Lysimachia thyrsiflora	.....	.....	4	5	.....	9
Eriophorium angustifolium	.....	.....	.....	9	.....	9
Salix candida	.....	.....	.....	8	.....	8
Bromus ciliatus	.....	.....	.....	8	.....	8
Spartina Michauxiana	.....	.....	.....	8	.....	8
Trifolium hybridum	6	.....	.....	.....	.....	6
Eupatorium perfoliatum	2	.....	.....	3	.....	5
Carex lanuginosa	.....	.....	.....	6	.....	6
Hordeum jubatum	3	.....	.....	.....	.....	3
Iris versicolor	.....	.....	.....	3	.....	3
Trifolium pratense	3	.....	.....	.....	.....	3
Plantago major	3	.....	.....	.....	.....	3
Asclepias incarnata	.....	.....	.....	2	.....	2
Sium cicutaefolium	.....	.....	.....	1	.....	1
Cicuta maculata	.....	.....	.....	1	.....	1
Pycnanthemum virginianum	.....	.....	.....	1	.....	1
Lobelia siphilitica	.....	.....	.....	1	.....	1
Erigeron philadelphicus	.....	.....	.....	1	.....	1
Cirsium muticum	1	.....	.....	.....	.....	1
Eleocharis palustris	sparse	dense	dense	sparse	sparse	.....
Eleocharis acicularis	sparse	sparse	.....	sparse	.....	.....
No. of species present	38	15	20	60	9	.....

Considering this table solely from the standpoint of abundance it is plain that the bulk of the vegetation belongs to a few species. This is more graphically shown by Plate XVIII which shows diagramatically the relative proportions of the fifteen leading species. It is noticeable that all but three of the fifteen leading species are sedges or grasses and it can be said that these three do not rank as dominants anywhere in the transect.

To make the summary more exact as to the nature of the vegetation the following table showing the percentages for the leading species may be arranged.

TABLE 3.

Total population of transect	52,377		
Total Carex species	33,989	or	63%
Total grasses	12,698	or	24%
Total Carices and grasses			87%
Total Carex stricta	21,127	or	40%
Total Calamagrostis canadensis	9,765	or	18%
Total for these two species			58%

The nature of the plant population and the quality of the product of the marsh in hay is easily understood from the figures. The great excess of sedges over grasses (sixty-three per cent to twenty-four per cent) and the high percentage (forty per cent) of the rank growing *Carex stricta* indicates that the hay value will be largely determined by the sedges. As far as the grasses are concerned the value is almost wholly due to the eighteen per cent *Calamagrotis canadensis* since the total of the remaining grasses (six per cent) is small.

For the purposes of a comparison of the families of chief importance numerically, the data may be grouped as follows:

TABLE 4.

	No. of species.	Total individuals.
Cyperaceae	13	34,024
Gramineae	11	12,698
Labiatae	5	2,838
Violaceae	2	314
Polypodiaceae	1	222
Compositae	9	191
Ranunculaceae	2	98
Alismaceae	1	98
Campanulaceae	1	82
Saxifragaceae	1	81

This table again emphasizes the great preponderance of the sedges and grasses and the relatively small numbers of dicots present.

Plant Associations

The general character of the vegetation being now established the question of the natural grouping can well be considered. For this purpose we may return to a study of table two. The numerical abundance of each species is there given for each association. But in considering the fluctuations in abundance it must be remembered that the associations are not of equal area. The data in the column for each association show what species are present and what the numerical proportions are. For study in connection with this table curves have been plotted showing the abundance and frequence of the principal species. For these the totals have been taken every fifty feet, instead of for the entire association as in table 2, hence the distribution is more exactly shown. On each fifty foot segment seventy-five quadrats were counted as previously explained and since in determining frequency the presence of a species in a quadrat is considered as a unit, the highest frequency possible for a fifty foot segment is seventy-five.

What may be called a Lycopus Caricetum occupies about five hundred feet of the south end of the transect. This is a border zone and is rather dry compared with the more central region. It slopes almost imperceptibly toward the center of the marsh. The soil borings taken showed there is more or less of the transported upland soil intermingled with the layer of humus and peat which in turn is underlaid by sand, gravel, and clay.

This border association is relatively rich in different species as shown in column one of table 2. *Carex stricta* and *Calamagrostis canadensis* lead in frequency and in numbers here as for most of the transect. Although clearly dominant for much of this association neither reaches its maximum development here. *Carex stricta* constitutes forty-two per cent by count and seventy-six by weight of the total vegetation and averages about eighteen inches in height. *Calamagrostis canadensis* consti-

tutes ten per cent by count and thirteen per cent by weight. In height it is about two and one half feet with rather few culms producing flowers. *Carex sterilis* is abundant but at its best development is seldom over eight inches in height and is a small low growing plant compared with the majority of the species present. Besides *Calamagrotis canadensis* the other grasses present are principally well developed plants of *Poa pratensis, Agrostis alba,* and *Glyceria nervata.* Together they constitute nineteen per cent by count and nine per cent by weight. These with *Carex Bebbii* and *Carex diandra ramosa* are present in sufficient numbers to be dominants in certain limited spots. Nearly all the other species given in column one of table 2 are subordinate species, few in numbers and much scattered.

This association is characterized by the development of the grasses above named and by the presence of a rather large number of dicots which, although constituting about one third of all the species present, amount to only about six per cent of the plant population. Of the dicots, *Lycopus uniflorus* is by far the most abundant, with *Carex stricta* leading the monocots. For convenience this association may therefore be called a Lycopus Caricetum.

Caricetum: The strip extending from the five-hundred-foot station to the seven-hundred-fifty-foot station on the transect shows a typical development of *Carex stricta.* This species is present on three hundred seventy-three of the three hundred seventy-five successive squares counted. It is of robust growth reaching on a level to the height of from twenty to twenty-eight inches and in several places showed very characteristically its stooling habit that gives it the name of tussock sedge. This stooling is quite characteristic of this sedge when it develops unhindered by secondary species and especially when the habitat is rather wet as in the case of development in or around ponds. They appear as clumps of closely packed culms at the base of which masses of underground branches and dead culms build up a rounded hummock of from six inches to three feet in diameter and often a foot or more in height. This species here con-

stituted seventy-five per cent by count and seventy-eight per cent by weight of the total vegetation.

Calamagrostis canadensis was abundant and frequent, and constituted twenty-five per cent by count and twenty-one per cent per weight of the total. This relatively low figure for weight is due to the fact that at nearly all points in this association the culms were weak and slender so that even in midsummer they were able to surmount *Carex stricta* in height only in a few patches.

From table 2 we see that but fifteen species were found here and that there is a marked decrease in the number of dicots. The line of contact of the Lycopus Caricetum and the outer edge of this association is marked in this changed character of the vegetation. There is also, seemingly, an increase of water content, a decrease of transported soil in the surface layers and a change to a pure blue clay substratum which is about three and one-half feet below the surface. As is shown by the plots in plate 19, the highest development of *Carex stricta* is reached in this part of the transect and for the convenience the portion from five hundred to seven hundred fifty feet of the south edge of the marsh may be called a Caricetum.

Calamagrostis Caricetum: From eight hundred feet to nearly fifteen hundred feet is found the wettest portion of the transect. *Carex aquatilis, Carex filiformis,* and *Carex Sartwellii* are limited to this portion of the marsh as is shown by curves in Plate III. *Carex stricta* is abundant but has dropped to twenty-two per cent by number and fifteen per cent by weight. The sedges, however, constitute sixty-five per cent of the total by count and about the same by weight.

Calamagrostis canadensis leads in totals for any one species. By count it constitutes thirty-four per cent and by weight thirty-six per cent. It is in this portion of the marsh that this species reached its best development and considerable patches of it reached the height of from four to five feet thus completely overtopping all other species.

There is, in this portion of the marsh, marked fluctuation within short distances in regard to the presence of the several

species. The plots of Plate XIX show that wherever *Carex aquatilis* and *Carex filiformis* develop there is absence of *Carex stricta and Calamagrostis canadensis.* The vegetation of this portion of the marsh is practically wholly of Carices and Calamagrostis and the association can be designated therefore, as a Calamagrostis Caricetum.

The water course shown to the left in the photograph (See Plate 20, Fig. 1) is about ten feet in width and but a few inches in depth. It cuts the transect at fourteen hundred fifty to fifteen hundred feet. Along and in this shallow water course there is a narrow strip in which *Scripus validus* and *Typha latifolia* are dominants. The species at this point are included in the list of those for the sedge and Calamagrostis association.

Lycopus Caricetum: A short distance to the north of this stream the transect crosses the canal shown in the photographs and immeiately north of this there is a very complex association extending about seven hundred and fifty feet between the stations at fifteen hundred feet and at twenty-two hundred and fifty feet on the transect. Sixty species are present and these are much intermingled. *Carex stricta* is a dominant over much of the area with a percentage of forty by count and fifty-two by weight. *Carex sterilis* becomes abundant but as in the border association it is too weak in habit to be ranked as dominant. *Calamagrostis canadensis* is well developed only in a few limited areas and shows a percentage of thirteen by number and seventeen by weight.

It is in this association that nearly all the species of Compositae are found, as well as the maximum development of *Aspidium Thelypteris, Lycopus americanus, Parnassia caroliniana* and other of the species listed for this association in table 2.

The habitat is a low ridge, elevated at its center about two and one-half feet above the lower portions of the marsh. Here the humus and peat is underlaid by sand. Evidently this was a sand bar or spit in the old lake bay and was probably the first to be occupied by the land plants. At the time of taking soil borings the water level lay below the surface of the sand which

came to within three feet of the surface while in the wet sedge and Calamagrostis association it was within seven and one-half inches of the surface. This is then a relatively dry portion of the marsh with at least a slightly better aeration and this is possibly the reason for the greater number of dicots (thirty-four species in all) many of which, as shown in table 2, are confined to this region. The plants typical of the wet portions are lacking here.

Caricetum: The transect cuts this low ridge and its characteristic vegetation diagonally and passes into a nearly pure but rather small association that is almost entirely composed of *Carex stricta.* This occupies a shallow but marked basin like depression about one hundred feet across. Here but nine species are present and their relative numerical proportions are given in table 2. This association terminates the marsh abruptly at this point.

Data for Typical Stations

A fuller view of the vegetation of the marsh can be gained by a study of the data collected in the following tables, each giving the population of a ten foot strip or station. These tables represent typical portions of the marsh and show exactly the grouping of species for each station. Since every other foot of the four inch strip was counted and each foot was divided into three quadrats the totals are for fifteen quadrats, or in all, an erea of one and two-thirds square feet. The totals for each station will be given in the first column and the frequence will be given in the second column. The presence of a species in a quadrat was considered a unit of frequency, hence, for example, fifteen under the head frequency means that the species named was present in each quadrat of the station in question.

Lycopus Caricetum. Twenty to Thirty Feet.

This is located close to the outer edge of the association. The humus and peat is about one and one-half feet deep but mixed with, and underlaid by, washed soil from the uplands.

The Species present.	Individuals.	Frequence.
Carex stricta	83	15
Carex sterilis	80	10
Poa pratensis	37	8
Agrostis alba	12	3
Lycopus uniflorus	10	5
Calamagrostis canadensis	9	4
Scutellaria galericulata	2	1
Equisetum arvense	1	1

Although *Carex stricta* is here dominant it is not stooling nor is it robust in habit. The vegetation is dense, a firm smooth turf is formed and it is distinctly a mixed association.

110 to 120 Feet.

This is in the Lycopus Caricetum association and is the region where *Carex Bebbii* reaches its maximum development.

The Species Present.	Individuals.	Frequence.
Carex stricta	129	14
Carex Bebbii	116	10
Lycopus uniflorus	13	3
Carex sterilis	5	1
Calamagrostis canadensis	4	2

140 to 150 Feet.

A spot where the vegetation is almost entirely of *Carex stricta.* It was not, however, stooling but stood smooth and even at the height of about eighteen inches. The *Calamagrostis canadensis* is weak and scattering.

The Species Present.	Individuals.	Frequence.
Carex stricta	237	15
Calamagrosits canadensis	31	11
Lycopus uniflorus	11	4

340 to 350 Feet.

This section is typical of the border association where *Carex stricta* has the least development. The species are scattered and intermingled giving a mottled covering. The vegetation is thin and sparse but the ground is completely occupied.

The Species Present.	Individuals.	Frequence.
Carex sterilis	106	7
Andropogon furcatus	46	7
Agrostis alba	43	8
Carex stricta	29	8
Comandra umbellata	27	9
Viola cucullata	16	6
Calamagrosits canadensis	14	3
Lycopus uniflorus	4	1
Glyceria nervata	3	1
Thalictrum dasycarpum	1	1

470 to 480 Feet.

A station close to the inner edge of the mixed association and one of the few points where *Glycria nervata* is dominant. The culms are fine and closely set.

The species present.	Individuals.	Frequence.
Glyceria nervata	167	14
Carex stricta	76	10
Lycopus uniflorus	19	3
Agrostis alba	10	3
Lycopus americanus	3	2
Calamagrostis canadensis	2	1
Lathyrus palustris	2	2

710 to 720 Feet. Caricetum.

Here *Carex stricta* reaches its maximum development and appears as described under general discussion of this belt. *Calamagrostis canadensis* culms are weak and subordinate at this point. *Eleocharis palustris* is abundant but weak and much scattered between and below the culms of the other species.

The species present.	Individuals.	Frequence.
Carex stricta	282	15
Calamagrostis canadensis	113	15
Eleocharis palustris	abundant	15

810 to 820 Feet. Calamagrostis Caricetum.

This is in the midst of the wet association, and was the point where *Carex aquatilis* reached its maximum development. The leaves stood on a level of about forty inches giving a coarse

sedgy aspect. *Calamagrostis canadensis* was in little evidence at any time during the year.

The species present.	Individuals.	Frequence.
Carex aquatilis	102	15
Calamagrostis canadensis	64	12
Carex filiformis	38	6
Carex Sartwellii	13	3
Sagittaria latifolia	3	2

860 to 870 Feet.

Here *Carex filiformis* reaches its maximum development and this is one of the wettest portions of the marsh. *Carex filiformis* with its slender wire like leaves does not form a dense vegetation and does not cover up the ground as do the other sedges of the strip. A heavy coat of moss covers the ground making it much more spongy than is the case elsewhere on the marsh.

The species present.	Individuals.	Frequence.
Carex filiformis	136	12
Carex aquatilis	66	12
Sagittaria latifolia	5	5

1180 to 1190 Feet.

A comparison of Plates 2 and 3 shows that *Carex Sartwellii* is a dominant form in this region from 1150 to 1350 feet and that *Carex stricta* is lacking. There is also a marked decrease of all other Carex forms. This ten foot section is typical of the region. *Carex Sartwellii* forms a dense and leafy vegetation about twenty-four inches high on the level. The habitat is slightly less wet than that of the purer *Carex aquatilis* association.

The species present.	Individuals.	Frequence.
Carex Sartwellii	150	14
Carex aquatilis	58	14
Carex filiformis	9	3
Calamagrostis canadensis	5	2
Eleocharis palustris	Abundant.	

1490 to 1500 Feet.

At this point the transect cuts the *Scirpus-Typha* association that occupies the shallow water course previously described.

The species present.	Individuals.	Frequence.
Carex filiformis	42	9
Carex riparia	29	9
Scirpus validus	12	6
Carex Sartwellii	8	2
Carex aquatilis	5	2
Calamagrostis canadensis	5	2
Typha latifolia	4	4

1640 to 1650 Feet. Lycopus Caricetum.

This is a typical segment of the mixed association and answers to the general description previously given. *Carex sterilis* can hardly be called a dominant because of its weak growth. It is seldom over eight inches in height and is hence overshadowed by most of the other species. The complexity of this whole association is well shown by the large number of species present on the fifteen quadrats here summarized. As many as eight species were present on a single quadrat four inches square.

The species present.	Individuals.	Frequence.
Carex sterilis	92	12
Carex stricta	53	12
Lycopus uniflorus	41	11
Calamagrostis canadensis	28	7
Glyceria nervata	27	5
Andropogon furcatus	22	2
Aspidium Thelypteris	13	12
Viola cucullata	8	7
Carex fusca	8	3
Mentha arvensa va. canadensis	7	3
Hypoxis hirsuta	5	3
Helianthus grosseserratus	4	4
Solidago uliginosa	2	2
Eriophorum angustifolium	1	1
Solidago neglecta	1	1
Eupatorium purpureum	1	1
Chelome glabra	1	1

1680 to 1690 Feet.

In this segment *Viola blanda, Solidago, uliginosa, Bideus frondosa,* and *Aspidium Thelypteris* all reach their maximum development. The vegetation is unusually sparse but the entire surface is fully occupied.

The Species Present.	Individuals.	Frequence.
Carex sterilis	93	10
Carex stricta	39	11
Viola blanda	20	7
Solidago uliginosa	16	7
Lycopus americanus	14	5
Bidens frondosa	14	5
Lycopus uniflorus	13	4
Aspidium Thelypteris	11	10
Parnassia caroliniana	11	8
Adropogon furcatus	9	3
Calamogrostis canadensis	9	4
Glyceria nervata	8	2
Salix candida	3	3
Viola cucullata	2	2
Mentha arvensis var	1	1
Lathyrus palustris	1	1

1780 to 1790 Feet.

This is another section typical of this whole association. *Lycopus uniflorus* here reaches its greatest development in numbers per quadrat. The various mints and compositae develope during late August and September and give a marked difference to the aspect of the flora in late summer.

The Species present.	Individuals.	Frequence.
Carex stricta	118	15
Lycopus uniflorus	74	13
Carex sterilis	49	8
Calamagrostis canadensis	20	6
Lycopus americanus	15	6
Mentha arvensis var	5	1
Salix discolor	3	2
Aspidium Thelypteris	3	2
Viola cucullata	2	2
Solidago uliginosa	2	1
Solidago neglecta	1	1
Caltha palustris	1	1
Thalictrum dasycarpum	1	1
Bidens frondosa	1	1
Polygonium amphibium var	1	1

1990 to 2000 Feet.

This is in the border of the mixed association. The vegetation is more dense and grass like.

The Species Present.	Individuals.	Frequence.
Carex stricta	70	13
Lycopus uniflorus	31	10
Calamagrostis canadensis	30	10
Carex sterilis	15	3
Glyceria nervata	12	3
Carex diandra var. ramosa	5	1
Viola cucullata	3	3
Parnassia caroliniana	3	3
Aspidium Thelypteris	2	2
Chelome glabra	1	1
Aster puniceus	1	1
Eupatorium perfoliatum	1	1

2140 to 2150 Feet.

A section of the association dominated by *Carex stricta,* with dense vegetation two feet high on the level and this is overtopped by the taller *Calamagrostis canadensis.*

The species present.	Individuals.	Frequence.
Carex stricta	63	12
Glyceria nervata	35	8
Calamagrostis canadensis	30	11
Carex sterillis	26	5
Galium trifidium	10	5
Scutellaria galericulata	4	4

2270 to 2280 Feet. Caricetum.

This gives the data for a portion that is typical of the association dominated by *Carex stricta,* which occupies the last one hundred feet of the transect.

The species present.	Individuals.	Frequence.
Carex stricta	215	15
Glyceria nervata	21	5

These more detailed discussions of typical ten foot sections of the various associations show that what has been called the Lycopus Caricetum, the Caricetum, or the Calama-

grostis Caricetum is in reality a complex of smaller associations, for in no section of ten feet do we find any one species excluding all others. The facts thus shown together with the curves given in plate 20, show the suddenness with which dominant species may change.

Among the species that may be considered as dominants there are further various degrees of development and domination. This is well shown in the fluctuations in the curves on plate 20. At station 75, although *Calamagrostis canadensis* was very abundant it was entirely overshadowed throughout the season by *Carex stricta* and did not even produce flowers and seeds.

Hay Production

To determine the relative weights of the various species in the composition of the hay the following plan was used. At intervals of fifty feet the vegetation growing on an area of five square feet was cut, sorted, labelled and tied into bundles. This was done during the last two weeks of August, 1907. The bundles were thoroughly dried under cover and then weighed. From the weights, per cents have been computed and the total hay production per acre estimated. The total is somewhat higher than would be obtained by methods of hay making, due to the fact that there was no waste.

These facts show the relative hay production of the various associations as well as the proportional weight values of the various species in the hay. The data as to weights are summarized in the following table.

Table 5.

	Lycopus Caricetum.	Caricetum.	Calamagrostis Caricetum.	Lycopus Caricetum.	Caricetum.
Av. weight per 5 sq. ft. in oz.....	6.3	9.7	13.	7.125	10.5
Estimated yield per acre in lbs.....	3,430	5,281	7,078	3,879	5,717
Per cent Carex stricta by weight.....	76	78	15	52	96
Per cent C. canadensis by weight. ...	13	21	36	17	.....
Per cent of other grasses by weight..	9	.....	.....	5	3
Per cent Carex aquatiltis by weight..	.....	.....	16	.....	.....
Per cent Carex Sartwellii by weight..	.....	.....	14	.....	.....
Per cent Carex filiformis by weight..	.....	.....	3	.....	.....
Per cent Carex riparia by weight.....	.....	.....	3	.....	.....

The total yield per acre is heaviest in the Calamagrostis Caricetum and least in the Lycopus Caricetum associations. The bulk of the vegetation by weight is composed of the two species *Carex stricta* and *Calamagrostis canadensis.* The former constituted forty-three per cent according to the data for the transect and the latter twenty-four per cent. Many tons of hay of this character are fed each year on the farms in the vicinity of Madison and many acres of this type of marsh land are utilized for pasturage. When pastured heavily it is noticed that the sedges are rather closely cropped especially in the early part of the season when they develop most rapidly and there is a general opinion that marsh meadows afford excellent grazing.

There seem to be no data on the digestibility of *Carex stricta.* Analyses by Bailer and Wilk (22) show 93.97 per cent of organic substance and 6.03 per cent ash of which 1.954 is $Si O_2$.

Storer (4) (1875) found that "bog hay" (composed of *Carex stricta*) taken from the barn gave analyses as follows:

Water	8.17%
Ash	5.54%
Albuminoids	6.88%
Carbohydrates and fats	45.99%
Cellulose (ash free)	33.42%

This bog hay showed very slight differences in comparison with meadow hay. He also noted that bog hay collected by hand in June was better than that mown later due to the fact that *Carex stricta* matures early in the season.

For *Calamagrostis canadensis* Jordan (6)p. 38 (1889) gives the results of the analysis of the water free hay as follows: Ash 5.70 per cent; Protein 10.19 per cent; Fiber 36.32 per cent. He states that it is the most valuable of lowland grasses and his tables show that it has a per cent of digestibility greater than that of timothy or red top.

Pammel (11) (1901) states that *Calamagrostis canadensis* covers a considerable area of lowland marshes in northern and central Iowa and is a well relished and valuable forage crop.

The other grasses of the transect aggregate but two or three

per cent of the total weight and the total weight of the herbaceous dicots is somewhat less.

The sedges characteristic of the wet portions are coarse and rough with the exception of *Carex Sartwellii* which makes a hay of fine texture.

The total weight percentage of the grasses is nearly equal for the Lycopus Caricetum and the Calamagrostis Caricetum with the yield decidedly in favor of the latter. What the nutrition value of the sedges in question may be should certainly be settled by analyses and experiments.

The mixture of *Calamagrostis canadensis* with the sedges gives manifest disadvantages for the utilization of the mixed crop as hay because of the different seasonal development of the species. It is evident that when *Calamagrostis canadensis* is ready for cutting, and this is the time when the hay is usually made, the sedges have passed maturity and are becoming dry. Few attempts have been made to improve marsh meadows of this type, but the high hay value and heavy yield of *Calamagrostis canadensis* suggests the feasibility of its use especially for the wetter portions of such marsh meadows.

Efforts to improve marsh hay lands have been begun in Wisconsin. The results have not as yet been published but they show that surface drainage accompanied by the tearing up and destroying the surface layer of moss, and the sowing of such grasses as *Phleum pratensis, Poa triflora, Agrostis alba* and *Calamagrostis canadensis* together with alsike give excellent returns.

Geographical Range of the Species

In considering the continental range of the species the plan of MacMillan (9a) is followed. The 95th meridian is taken as the line between the eastern and the western half of the continent and the 45th parallel N. latitude as the line dividing the northern from the southern region. The distribution of a species with reference to these lines determines the range. In most cases the range of species here given is taken from MacMillan (1892). The following is a tabulation of the continental

range extent of the species listed in table 1 with the exception of the mosses.

TABLE No. 6.

Range of Species.	Crypto-gams.	Monocots.	Dicots.	Totals.
N S E W	2	16	13	31
N E W	.....	8	15	23
N S E	.....	4	4	8
N E	1	1	6	8
N W	.....	2	1	3
S E	.....	5	17	22
S E W	.....	1	6	7
S W	.....	0	0	0
Introduced species	.....	.....	.....	4
	.....	.....	.....	106

It is noticeable that the plants which belong to the N S E W range are about evenly divided between monocots and dicots and that in totals there is little difference between the three range groups N S E W, N E W, and S E.

Summarizing the above table and securing the total number of species of the four ranges N, S, E, and W we have:

TABLE No. 7.

	Monocots.	Dicots.	Totals.
Northern	31	39	70
Southern	26	40	66
Eastern	35	71	106
Western	27	35	62

This shows that as a whole, the species of this marsh meadow are decidedly eastern with little difference between the other ranges. MacMillan finds that the vegetation of the Minnesota River Valley is decidedly southern and eastern in range and he points out that this is the result of the "geological, topographical, and hydrographical southeasternness of the valley together with biological factors concerned with continental pressure tensions."

If we now compare the number of species from the north with those from the south, we find that the plants of N E W or those of distinctly northern range lead in number of species throughout the transect except in the complex Lycopus Carice-

tum. Those of the N S E W range fluctuate considerably in number. This is shown in the following table.

TABLE No. 8.

	Lycopus Caricetum.	Caricetum	Calamagrostis Caricetum.	Lycopus Caricetum.	Caricet
Introduced species	3	.....	.....	1	.....
Species of N S E W range	10	5	7	20	4
Species of N S E range	2	3	3	5	1
Species of N E W range	12	8	9	16	4
Species of N W range	3	.....	.....	.....	.....
Species of S E range	5	.....	1	12	.....
Species of N E range	1	.....	.....	5	.....
Species of S E W range	2	.....	.....	1	.....
	38	15	20	60	9

The wetter middle portions of the transect are occupied by species of limited range only and in these the northern distribution is strong. In marked contrast is the drier border portion with large numbers of species and a marked mixing of range elements.

The geographical distribution of the fifteen species most abundant on the whole transect are tabulated below.

TABLE 9.

Per cent of total Abundance.		Range.	
40	Carex stricta	N S E	Ex. Continental Europe.
18	Calamagrostis canadensis	N E W	Ex. Continental Siberia.
6.4	Carex sterilis	N S E W	Not Ex. Continental.
6.3	Carex Sartwellii	N E W	Ex. Continental Asia.
4.4	Lycopus uniflorus	N E W	Ex. Continental Asia.
3.8	Carex aquatilis	N E W	Ex. Continental Europe.
3.7	Carex diandra var. ramosa	N E W	Not Ex. Continental.
2.9	Glyceria nervata	N S E W	Not Ex. Continental.
2.5	Carex filiformis	N E W	Ex. Continental Eurasia.
1.1	Agrostis alba		Introduced.
1.0	Carex Bebbii	N S E W	Not Ex. Continental.
0.6	Poa pratensis	N W	Ex. Continental.
0.5	Scutellaria galericulata	N E W	Europe, Asia, Japan, Africa.
0.5	Andropogon furcatus	S E	France.
0.4	Viola cucullata	N S E W	Not Ex. Continental.

While *Carex stricta* is to be considered as a member of the northern element its distinctive range is N S E and as such it belongs to a region that has few representatives in the marsh.

The N E W element has seven members in this list aggregating about forty per cent of all the vegetation. It also leads in number of species in every association except the mixed association. The high precentage of northern, eastern, and western elements in such a marsh meadow is clearly evident.

It is to be noted that the western element present here is also a northern element and that there is an absence of any element that is S W or even N S W. These facts apply to the vegetation as a whole according to MacMillan's analysis of the Minn. River Valley, the cause of which is to be found in the "southeasternness" of the area as a whole.

While the vegetation of this marsh meadow shows this influence in the presence of a strong eastern element there is in the important northern element an evidence of other influence. The abundance of species of northern as well as eastern range, especially in the wetter portions of the marsh emphasizes the influence of the glacial period. Five of the six principal species found in the marsh meadow and which comprise 72.5% of the total vegetation are of extra continental range, a fact that points to a common northern center of development.

It should be stated in this connection that in regard to the species here under discussion exact data are lacking concerning the geographical range with the varying habitats and the corresponding fluctuations in the vegetative and the reproductive vigor of the species. These facts must be determined as a basis for the satisfactory understanding of the abundance of a plant in any given locality. Plants that are no more highly specialized than are those here under consideration are capable of developing under a variety of conditions climatic as well as edaphic. In a general way the distribution of the species is known but statistics as to the relative abundance and centers of greatest development are lacking and the data at hand can not hence be shown to be definitely related to known climatic or edaphic conditions.

Distribution of Marsh Meadows in North America

Of equal importance is the more complete study of the geographical distribution of the physical conditions represented in marsh meadows with reference to the climatic and edaphic conditions that are operating. The literature reviewed above indicates the inadequacy of the data available.

Transeau (16) (1903) has sketched a map of the "bog societies" of North America. He recognizes that these are intimate relationships between "bog societies" and "swamp societies or drained swamps." In his discussion he pays no special attention to the marsh meadow type which is however evidently included in his "drained swamp." He concludes that in general the belt of bog societies corresponds on the north to the border of the forest belt and that bog societies are typical of the colder portions of North America. He notes that an examination of a diagonal area extending northwest and southeast through central and southern Wisconsin shows a noticeable thinning out of the bog societies in its east and west extension. This Transeau considers as evidence of a transition from a forest to a prairie region.

It should be noted further, that this is a belt in which marsh meadows are especially developed and that these are evidently more closely related to prairies than are "bog societies."

Transeau considers that "under present conditions the bog societies cannot compete with marsh plants in the possession of newly exposed undrained areas, but decides that neither presence of peat, depth of water, or manner of seed dispersal can account for the preponderance of marsh plants. His conclusion is that the bog plants are remnants of the bog and tundra vegetation that bordered the glacial front and that in the capture of newly exposed undrained areas swamp societies are now successful because they are made up of more southerly forms and are hence the normal hydrophytic vegetation of the present climatic conditions.

In regard to this point it is be be noted, however, that the ranges of the principal species found on the marsh meadow in

question show as wide a distribution especially to the north as do the bog plants.

The facts of distribution seem to indicate that the deciding factors in this case are chiefly edaphic rather than climatic and that the conclusions noted above as reached by Chamberlain and also by Whitson and Jones that the heath group is intimately connected with an merges into the forest, while the marsh type leads to the prairies is the more nearly correct.

Horizontal Stratification

The plants of the marsh show also a characteristic horizontal stratification that is generally considered as due to the light requirements of the different species.

Recent investigations of Yapp (24) (1909) show that there are marked differences in the evaporation at the various levels and that this is an important factor in influencing the aerial structure of the various species. Close to the ground and shaded by the sedges and grasses there is a rather compact carpet like growth of moss covering most of the marsh. In the mixed associations low growing plants like *Viola blanda* and *Parnassia caroliniana* seem to also thrive in spite of considerable shading by taller forms. Over much of the area *Eleocharis palustris* grows with slender weak culms seldom more than eight inches in height and which are abundantly scattered among the taller plants. This is true also of *Carex sterilis*.

There is further also what may be called a seasonable development which results in the successive ascendency of different species and a corresponding change in aspect of the whole flora. At several points in all the association where *Carex stricta* and *Calamagrostis canadensis* were nearly evenly balanced in number and frequence this was very clearly shown. In June and early July the Carices flower and reach their maximum development completely overtopping Calamagrostis, but in July and August the latter develops and forms a leafy stratum above the sedges and in some cases almost entirely masks the sedges below. The various Labiatae and Compositae also develop late in summer or in the early autumn and by rather profuse

branching and flowering give a new aspect to those portions of the marsh in which they occur.

Adaptations

The adaptation of the plant members in so far as it affects their relations to each other and their consequent combinations in plant associations forms a most important phase of the study of such a population as we are considering. The vegetation is almost entirely herbaceous and the majority of the species are perenials.

The various species of Carex found, have, in general, a similar and characteristic form of growth, both in their underground and aerial portions. We may take as a type *Carex stricta*. In the ground there is a well developed root stock system. The wire like rootstocks grow about six inches below the moss layer and below the roots of many of such secondary species as may be present. The terminal buds turn up and develop aerial branches in the form of clumps of culms. By continued growth and branching from the bases of their culms a thick dense cluster of culms are produced. When a culm produces fruit it dies. Two types of growth and branching of rhizomes are thus to be distinguished. One for spreading and reaching new territory and the other for the immediate production of aerial culms. A root stock grows for a considerable distance, often to a length of more than a foot, then its terminal bud sends up aerial leaves and the formation of a tussock is begun. Further creeping rhizomes arise from the tussock and thus the two types of branching provide for vegetative spreading and for leaf and flower production.

Two types of roots are developed. Long, cylindrical, mostly unbranched roots develop from the area of short compact internodes at the base of the culms and push downward into the cold saturated peaty soil to a depth of six or eight inches. These are essentially soil roots. Fine fibrous roots also develop at the base of the culms but grow upward to the surface where they form a mass of finely divided rootlets.

The various species differ in the number, size, and position

of root stocks and roots, and in the size of the clumps of culms. *Carex aquatilis* is large and coarse, has few culms in a clump and produces most definitely the two kinds of roots (See Plate XXI. The rootstocks and soil roots often grow to be a foot long and both are provided with aeriating tissue. The soil roots have no branches. The method of branching is the same as noted for *Carex stricta*. *Carex riparia* is similar but is still coarser in growth and produces roots from the rootstock (See Plate XXII. The rootstock of *Carex Sartwellii* does not branch much laterally (See Plate XXII).

Compared with these sedges *Calamagrostis canadensis* has a fine and much branched underground rootstock system which lives nearer the surface. The roots are all finely fibrous and the branching that results in aerial shoots is loose.

The general adaptations of these plants have been repeatedly pointed out. The conditions require a superficial or at least a shallow root development. This limits the aerial growth and forces exposure to sun and winds. The herbaceous habit is adapted to the seasonal extremes of aerial conditions. The rhizomes provide for spread and for perpetuation. The abundance of root development with air chambers in roots, rhizomes and scales is adapted to the water and soil conditions.

Yapp (24) (1909) has pointed out the unsatisfactory state of our knowledge of the adaptations of xerophytic swamp plants. His studies of the evaporation at different levels show a marked corellation of the stratification of the plants with the varying amounts of evaporation. The xerophytic leaf structures develop in the upper levels while the mesophytic are below in the zone of decreased evaporation. He considers that the aerial structures are adaptations to the extremes of evaporation that exist.

The view that swamp xerophytes are results of the physiological dryness of the swamp habitat is somewhat in question as Clements (21) (1905) emphasizes. He considers that the xerophytic structures of such plants are not due to presence of humic acids in the soil but that these features "are due to the persistance of stable structures which were developed when these species were growing in xerophytic conditions."

The plants that give character to this marsh vegetation possess, we may say, the same general appearances and habit of growth and hence should be competitors. Yet if we analyze their adaptations further we are forced to give up any such general conclusion.

To illustrate: There are, in spite of the same general grass-like habit of growth, marked anatomical differences already mentioned between *Carex stricta* and *Calamagrostis canadensis.* Yet as shown in plate 19 there is a tendency for both to occupy the same territory. One would say at first thought that these two species are close competitors yet the mode of root and rhizome growth, the vertical stratification, and the seasonable development really provide two different environments for the two plants altho they may stand side by side.

A plant society may be not so much a collection of plants of various species which are adapted to the same conditions as an association of species which are adapted in a different fashion to the same locality. This fact has not been sufficiently recognized in many ecological studies, but has been most clearly pointed out by Yapp in his studies of marsh vegetation in England.

One of the most difficult points in solving the problems of plant adaptation in this marsh meadow is the mutual exclusiveness of *Carex stricta* and *Carex aquatilis.* Large numbers of rhizomes, roots, and vegetative shoots were examined as to structure and general habit of growth. This afforded no evidence as to why there should be such a marked differential distribution with such apparently slight differences in the environment. The solution does not seem to be with the differences in the vegetative habit or in the anatomy. Yet it must be said that we know very little of the root and rhizome habit of the sedges and grasses. We need detailed studies of various root and rhizome systems and the influences which determine them.

The evidence afforded by the distribution of these species in the marsh meadow studied seems conclusive that *Carex aquatilus, Carex riparia, Typha latifolia, Scirpus validus* and *Carex*

filiformus were the first of the species now present to gain a foothold. These species occupy the wetter portions of the marsh. *Carex stricta* is evidently a more recent arrival and altho of general distribution in the marsh yet reaches its greatest development between the wetter portions and the border zone. The range of *Carex stricta* is N S E, while that of *Carex aquatilis* is N E W. This indicates that *Carex aquatilis* is more northern in its climatic requirements.

The conditions of competition in such a dense population already so fully occupying the space affords a most interesting field for the study of development. Practically every inch of the ground is occupied and hence the question of seed dispersal is of little importance in the spread of species. The struggle is chiefly between species very similar in structure and adaptation. This struggle is severe beneath the ground for the rhizome development admits of steady persistent spread and gives rather permanent possession even after the most favorable conditions have ceased to exist. Yet it is noticeable that there is a definite grouping of species with definite areas of best development and marked zones of contact. Sligth differences in moisture content, in soil composition, and slight elevations or depressions are associated with change of species. In a habitat with such uniform conditions as is here found and with so many species of similar structure, slight differences in environment are correlated with the individual peculiarities of the various species most strikingly.

Influence of Water Level

It is universally recognized that the amount of water in a soil and the level at which the ground water stands are important direct factors in determining the character of the plant life which is present.

A series of experiments was carried out to test the influence of this one factor and to determine whether there is a corellation between depth of the water table and the root and shoot development. Seeds of the species that are dominant in the marsh meadow above described were not available when these

experiments were taken up and hence in view of the problems involved in the growth of more valuable forage plants on these marsh meadows, the following species were used for the tests: Alsike clover *(T. hybridum);* red-top *(Agrostis alba);* timothy *(Phleum pratense);* and *Calamagrostis canadensis.* The latter as has been shown is the most important grass of the marsh meadow. The other species are more or less in use in the attempts to improve marsh meadows.

For the experiments six galvanized iron cylinders three feet in diameter and four and one-half feet in height were selected from the soil cylinders in use in experimental work carried on in the greenhouse of the Department of Soils of the Agricultural College. These particular cylinders had previously been filled with muck soil obtained from marsh land and hence closely duplicated the soil conditions of the marsh meadow in question. Strips of galvanized iron sheeting were set edgewise into the soil in such a manner as to divide the surface area of each cylinder into four equal segments. A cylindrical basket made from eight-mesh galvanized wire screening was placed in the center of each of these areas. These baskets were six inchs in diameter and from two to two and one-half feet in length. In sinking the baskets the soil was carefully sifted and gently tamped in and around each basket to prevent checks or pockets. Thus there was provided a total of 24 baskets, 4 in each cylinder and each basket imbedded in the center of one quarter of the surface of the cylinder.

The bottom of the cylinders were covered with several inches of broken tile which formed a sort of reservoir beneath the dirt. To regulate the height of the water table, glass tubes were fitted by means of rubber corks into a tap connection at the bottom of the cylinder. The glass tubes were arranged to stand erect at the side of the cylinders and hence to act as water gauges. The height of the tube attached to a cylinder determined the position of the water table within that cylinder for any excess would flow from the top of the gauge until the level sank to the height of the gauge.

The cylinders were prepared between Feb. 7 and 14, 1910.

Water was applied to the surface frequently until Mar. 25 without any planting in order to secure complete adjustment of the water levels. In March 25 seeds of alsike, red-top, and timothy were sown, each species in one of the quarter areas of each cylinder. It was impossible to secure seeds of the blue joint and so the areas designed for this species remained vacant until Apr. 12, when rhizomes were transplanted from the marsh meadow to the cylinders.

Each of the four species thus occupied one quarter of the surface area of each cylinder and was enclosed in a wire basket placed as described above. The water gauges were adjusted to allow the water to stand as follows:

Cylinder No. I, at the surface of the soil.
Cylinder No. II, 4 inches below the surface of the soil.
Cylinder No. III, 8 inches below the surface of the soil.
Cylinder No. IV, 12 inches below the surface of the soil.
Cylinder No. V, 24 inches below the surface of the soil.
Cylinder No. VI, 30 inches below the surface of the soil.

The water used was from the pipes of the green house and was applied by means of a hose to the surface of the cylinders. In cylinders I and II the surface was flooded for about 30 minutes after each application. Evaporation would lower the water level in cylinder I so that much of the time it was slightly below the surface. During the greater part of the time the experiment was run there was no difficulty in keeping the water-level of the gauges. During the hot weather of June the water table usually fell somewhat below the level of the gauges between the applications of water. At first the seedling in cylinders I and II made the best progress. Aside from this there was no decided differences noticeable until May 6 when the following notes were recorded.

Timothy.

Cylinder I, 2¼ inches high, yellowish, doing poorly.
Cylinder II, 6 inches high, green, doing well.
Cylinder III, 7½ inches high, green, doing well.

Cylinder IV, 10 inches high, green, doing the best.
Cylinder V, 6 inches high, about as in II but greener.
Cylinder VI, 4 inches high, green but small in size.

Red top.

Cylinder I, 2½ inches tall, yellowish.
Cylinder II, 2½ inches tall, not so yellow as in I.
Cylinder III, 3½ inches tall, green, doing well.
Cylinder IV, 4½ inches tall, green, best of all.
Cylinder V, 4½ inches tall, green, doing well.
Cylinder VI, 2 inches tall, green, slow in growth.

Blue joint.

In all cylinders the culms were from one to one and one-half feet in height and showed no marked differences in height and color. Certain plants in all the cylinders showed parasitic effects of the fungus Sclerotium rhizodes.

Alsike clover.

Cylinder I, 1½ inches tall, green, leaves small.
Cylinder II, 4 inches tall, green, leaves larger than in I.
Cylinder III, 4 inches tall, green, poorer than in II.
Cylinder IV, 3 inches tall, green.
Cylinder V, 3 inches tall, green.
Cylinder VI, 2½ inches tall, green, poor growth—sparse because of poor germination.

All the species except Calamagrostis showed at the end of this six weeks growth marked differences in appearances in the various cylinders. All did poorly at the extremes of the water levels.

The data taken June 1st. were as follows:

Timothy.

Cylinder I, 5½ inches tall, yellowish, erect, poor.
Cylinder II, 9 inches tall, yellowish, erect, poor.
Cylinder III, 12½ inches tall, yellowish, lodging.

Cylinder IV, 13 inches tall, green, lodging.
Cylinder V, 11 inches tall, green, lodging.
Cylinder VI, 11 inches tall, dark green, less lodging.

Red top.

Cylinder I, 5 inches tall, yellowish, doing poorly.
Cylinder II, 5½ inches tall, yellowish, doing poorly.
Cylinder III, 7 inches tall, green, erect.
Cylinder IV, 8 inches tall, green, lodging.
Cylinder V, 12 inches tall, green lodging.
Cylinder VI, 10 inches tall, green, lodging.

Blue joint.

Cylinder I, 20 inches tall, yellowish, in blossom.
Cylinder II, 18 inches tall, yellowish, in blossom.
Cylinder III, 21 inches tall, light green, in blossom, best of all.
Cylinder IV, 20 inches tall, light green, in blossom, doing well.
Cylinder V, 20 inches tall, green, in blossom.
Cylinder VI, 20 inches tall, green, no blossoms.

Alsike Clover.

Cylinder I, 5 inches tall, small fine leaves, green.
Cylinder II, 8 inches tall, large leaves.
Cylinder III, 8 inches tall, green and doing well.
Cylinder IV, 8 inches tall, green and doing well.
Cylinder V, 10 inches tall, green and doing well.
Cylinder VI, 9 inches tall, good but sparse.

The conditions of relative vigor that existed at the end of six weeks growth were in general still preserved. Continued growth by all species was made in all the cylinders and this was somewhat greater considering the time involved. The period of three weeks since the previous notes were taken was a period of rather rapid growth which was more apparent in cylinders with low water table.

During the latter part of June the heat in the green house became rather excessive for the best development of these plants. By July 1st it was evident that the crop had reached its best development under the conditions and so from July 9 to 11 the final data were taken on the aerial development and the baskets were dug out to determine the facts concerning the root development.

The cylindrical baskets were easily removed. The tops of those plants growing within the area included in a basket were of course attached to the main roots included within that basket. In a few cases roots extended slightly below the bottom of a basket and in those cases it is possible that some of the finer and deeper rootlets were broken off in the process of removing the baskets.

A fine stream of water from a hose was used to wash the dirt from the roots within the basket and the development of the roots was studied as to depth of penetration and degree of development at the various levels.

In all cases the plants growing within the area of the baskets were typical of those of that segment. The measurements of root and shoot development were taken from the surface of the ground. The culm measurements were taken to the tips of the longest leaves.

The final data on the plants are as follows:

Timothy.

Cylinder I. Roots 5½ inches, dense mass of fine roots in upper 1½ inches of the soil. Stems 6½ inches, leaves yellowish and sparse.

Cylinder II. Roots 24¾ inches, dense in upper 4 inches. Stems 14½ inches, leafy and yellowish green.

Cylinder III. Roots 27 inches, denser in upper 6 inches. Stems 19 inches, leafy, green, fruiting.

Cylinder IV. Roots 24 inches, dense in upper 6 inches. Stems 26 inches, leafy, green, fruiting.

Cylinder V. Roots 16 inches, denser in upper 6 inches. Stems 11 inches, green and leafy.

Cylinder VI. Roots 24 inches, as in V. Stems 11 inches, more erect than in V.

This development in relation to the water and soil levels is graphically shown in Plate XXIII. In this experiment we note that timothy barely existed in cylinder I where the root and shoot growth was decidedly inhibited. Here the upper 1½ inches of soil was practically one mass of fine roots. The best general development was in cylinders III and IV where at least one-half of the length and one-fourth of the total root mass was in the water. In cylinders V and VI with the water level close below the tips of the roots and the roots growing in soil of high capillary power the development of roots was somewhat less and that of the stems decidedly less than in cylinders II, III and IV.

Red top.

Cylinder I. Roots 17 inches deep, mostly in upper 7 inches. Stems 7 inches, yellowish, poor leaf development.

Cylinder II. Roots 24¾ inches deep, well developed below water level. Stems 15 inches, leafy but yellowish.

Cylinder III. Roots 29½ inches deep. Stems 19 inches.

Cylinder IV. Roots 24 inches deep. Stems 15 inches.

Cylinder V. Roots 23 inches deep. Stems 17 inches.

Cylinder VI. Roots 25½ inches deep. Stems 17 inches.

Red top grew poorly in cylinder I with the water at the surface and it made its best growth in Cylinder III with the water 12 inches below the surface of the soil. There were no marked differences in the other four cylinders. In II, III, and IV the roots grew well in the free soil water and the general development was nearly the same as in V and VI where the roots developed above the water table. See Plate XXIII.

Blue joint.

Cylinder 1. Roots 18 inches, densest at surface. Culms 20 inches, fruiting, leafy, yellowish.

Cylinder II. Roots 26 inches. Culms 20 inches, fruiting.

Cylinder III. Roots 33 inches. Stems 24 inches, fruiting.

Cylinder IV. Roots 30 inches. Stems 24 inches, fruiting.
Cylinder V. Roots 25½ inches. Stems 20 inches, fruiting.
Cylinder VI. Roots 25 inches. Stems 20 inches.

These blue joint plants were grown from rhizomes transplanted from the field and not from seed. This may account in part for the rather uniform results (See Plate XXIII). This species made a good growth in cylinders I and II but the foliage was yellowish. It fruited in all of the first five cylinders and showed little differences in the root development in all but the 1st cylinder.

Alsike clover.

Cylinder I. Roots 6 inches. Stems and leaves 8 inches.
Cylinder II. Roots 24½ inches. Stems and leaves 9 inches.
Cylinder III. Roots 25½ inches. Stems and leaves 11 inches.
Cylinder IV. Roots 25 inches. Stems and leaves 9 inches.
Cylinder V. Roots 21 inches. Stems and leaves 15 inches, in blossom.
Cylinder VI. Roots 21 inches. Stems and leaves 9 inches.

This plant did poorly in cylinder I. The main roots were short and thickened and bore many branches close to the surface. The aerial development was mostly leaves of good color but noticeably small. The different water levels in the other cylinders did not cause any marked differences except that plants in cylinder V were the only ones to produce blossoms.

Discussion of the Experiments

The final results for all the species are shown geographically in Plate XXIII with the depth of root growth and the height of the shoot development drawn to a scale in reference to the surface of the soil and the water level. This shows that under the conditions given the roots of all the species tested can extend below the water level and make a vigorous growth in the water saturated soil. The best general plant development was in cylinders III and IV where the roots had a zone of soil 8 and 12 inches thick above the water table. In these the root develop-

ment was more extensive than was the case in cylinder V and VI where there was much deeper water tables. There is however a marked uniformity in the root development in cylinders II, III, IV, V and VI irrespective of the wide differences in the water level.

In cylinder I all the plants made a dense mat like growth of roots in the surface two inches of soil below which there was relatively little root growth. While red top and blue joint sent roots down into the water to a depth of 17 and 20 inches these roots did not branch much. In the other cylinders there was no marked zonal distribution of the roots although there were relatively more roots in the upper layers.

While removing the baskets it was noted that the soil in cylinder V had a poorer consistency (structure) than did that of the other cylinders. The soil was more compact and broke up into hard compact chunks. In this regard the conditions were more unfavorable for root development in this cylinder. In all other cylinders the soil was loose and granular in texture.

The greatest differences between the four species in any one cylinder was seen in No. I. Th blue joint and red top showed a marked ability to develop with the water level at the surface but this was unfavorable for their best development.

In cylinder VI none of the roots extended to the water level which was 2½ feet below the surface and the growth that each species here made was surpassed in at least one other cylinder. This indicates that the water level suitable for the maximum development has been passed and that no better growth can be expected with a still lower water table.

Ten Eyck (20) (1904) has studied the root development of a number of grasses grown in the field. He was not concerned with the influence of various water levels and he did not examine any of the species tested here. He found that each species which he studied has a specific and characteristic root development and that the perennial grasses produce as a rule a large number of fibrous roots near the surface while at the same time the root system penetrates to a considerable depth, as for example, Kentucky blue grass, 4 feet; *Andropogon furcatus,* 6½

feet; *Bromus inermis* 5½ feet. None of the species tested made in the time this experiment was run such an extensive root development as Ten Eyck found.

The conditions under which these experiments were run do not exactly duplicate field conditions. The soil in the cylinders was without doubt warmer than the soil at similar depths in the field. The repeated application of water to the surface gave opportunity for better aeration. The plants were grown free from competition with other species.

This kind of experimental work, however, sheds some light on plant requirements and under such control, species can be tested in reference to known conditions both with and without competition and the data thus obtained can be corellated with the facts ascertained in the field.

The results of these experiments suggest the following conclusions for the species tested:

1. There is sharp correlation between root and shoot development.

2. There is poor development when the water level is constantly at the surface.

3. There is a marked development of roots in water soaked soil when the water level stands at 4, 8, and 12 inches.

4. A high water table (not above 4 inches) is not prohibitive to the growth of these plants, but rather is favorable for the best growth.

It would be of interest to test the Carices in a manner similar to that used in the experiments just described. None of these marsh Carices about Madison set seed during the spring of 1910 hence experiments of this kind have been deferred. There is evidence however that water level is not the sole factor determining the distribution of these plants.

The ditch shown in Plate XX was dug in the summer of 1906. In constructing it large masses of sod were often transferred from the level of the marsh to the top and sides of the ridge of dirt thrown out. The photo shown in Plate XX shows this condition. In 1910 this ridge of newly exposed soil was well covered with such plants as *Salsola Kali* var. *tennifolia, Oenothera*

biennis, Melilotus alba; Helianthus grosseserratus and *Salix amygdaloides.* These are species that are not found in the marsh meadow. At many places however *Carex stricta, Carex aquatilis, Carex riparia* and *Calamagrostis canadensis* are flourishing and spreading from the sod transplanted to the tops and sides of the ridge five or more feet above the highest level of ground water and in direct competition with the recent incomers named above. In such locations these species are not provided with their natural water conditions but are able to grow and spread when thus transplanted.

The statistical study of the transect shows that the blue joint develops best between the border zone and the central wetter zone where the water level varies during the summer from about 4 to 20 inches in depth. The tests in the cylinders give the same result showing that this species thrives well with the water level at 4, 8, 12 or 24 inches below the surface.

The field study showed that red top, timothy, and alsike were confined to the border zone where the water level was at least three feet in depth throughout most of the growing season. In this case the results of the field study do not agree with the results in the cylinder experiment—thus indicating that other factors besides water level are concerned in the case of these species.

The Evolution of the Marsh Meadow

As to the evolution of such a marsh as a whole the general principles of the physiographic theory as especially emphasized by Woodward (10) (1894) and later by Cowles (14) (1901) and Harshburger (19) (1904) are of considerable significance. Drainage has brought about the change from a water habitat to a condition such as is found on the marsh at present. More complete drainage will produce marked changes. In the vicinity various gradations coincident with degrees of drainage exist, showing progress toward a type of wild hay meadow with more grasses and less sedges.

Classification of the Marsh Meadow

Warming (25) (1909) classifies the plant formations closely related to the marsh meadow described above as follows:

A. The soil is very wet and the abundant water is available to the plant (at least in class I). The formations are therefore more or less hydrophylous.

Class I. Hydrophytes (of formations in water).

Class II. Helophytes (of formations in marsh).

1. Reed swamp formations.
2. Bush swamp formations.

B. The soil is physiologically dry; i, e, contains water that is available to the plant only in slight extent. The formations are therefore essentially composed of xerophylous structures.

Class III. Oxylophytes (formation on acid soil).

1. Low moor.
2. Grass heath.
3. High moor.

F. Soil and climate favor the development of mesophylous formations.

Class XIII. Mesophytes.

A. Communities of grasses (used in a wide sense to include Gramineae Cyperaceae, Juncaceae, etc.).

1. Arctic and Alpine not grassland.
2. Meadows.

It should be noted that among the species of the reed formation Warming includes *Carex stricta, Carex aquatilis Carex riparia* and *Carex filiformis* which are dominants in the marsh meadow described above, and also *Phragmites communis, Scirpus lacustus* and *Typha latifolia* which are present but less abundant.

Warming recognizes that there can be no sharply defined ecological classification of plants on account of the gradations between the types. It is to be noted, however, that Warming separates widely the meadow and the meadow moor making the former mesophytic and the latter oxylophytic and in this respect his classification is not satisfactory for our Wisconsin

conditions. Here, as has been stated above, the typical marsh meadows develop in glaciated areas underlaid by limestone and the soil of the marsh meadows is very slightly if at all acid. In other respects our marsh meadow agrees with Warming's low moor.

Evidently there are meadow moors of somewhat similar vegetation developing under both acid and neutral conditions. The latter is the type here under consideration. It differs sharply in this respect from Warming's low moor for which he assumes that the abundance of humous acids determines the general xerophytic character of the vegetation. The vegetation of our type agrees with Warming's characterization of the low moor. It differs in the presence of abundant Calamagrostis canadensis even in quite wet parts.

It is probable that acidity is a factor of considerable importance. The conditions in the low areas of the extreme eastern portion of Dane county and the western portion of Jefferson county, not over 35 miles east of the region here considered, indicate that where acidity prevails the vegetation conforms much more nearly to Warming's oxylophytic series and leads up rapidly to forest conditions. It is also to be stated that in large swamp areas the soil is often neutral around the margin and strongly acid in central portions and under these conditions there is transition from marginal meadow-moor formations to sphagnum and tamarack formations in the central portion.

The data on the plant succession leading to the marsh meadow in question together with the general conditions that exist in the marsh areas near Madison also indicate that the conditions are not correctly represented in Warming's oxylaphytic series. Here the succession has led through hydrophytic formations and reed swamp formations to marsh meadows which in turn give way to meadows and low prairies or possibly to a bush swamp formation leading to low lying timbered areas.

In the poorly drained areas of the state underlaid by sandstones and granite the succession leads to typical bog formations (the Heath Group of Chamberlain) and to tamarack and spruce forests. The latter are related to the forest belt.

The marsh meadow here described seems to agree in its gen-

eral characteristics most nearly with those described by Lewis, by Yapp, and by Smith as occurring in various parts of Great Britain, also with Warming's meadow moor of northern Europe, and a type described by Bailer and Wilk as present in Austria. Lack of data as to frequency and abundance of the species which make up the population of these European marsh meadows make detailed comparisons impossible. For a more complete understanding of the principles determining the vegetation of the marsh meadow it is of fundamental importance that we should have more exact data concerning the geographical distribution of marsh meadows, the range of variations in the composition and vigor of the plant population and the corellation of these facts with the geographical, edaphic and biological factors that are of influence.

Summary.

The habitat of this wild hay meadow is that of a nearly level and poorly drained marsh with a peaty surface stratum.

The vegetation is prevailingly of sedges and grasses of which *Carex stricta* and *Calamagrostis canadensis* are dominant species.

There is a marked grouping of species with few species in the wetter portions and a more complex grouping on the higher and drier portions.

The vegetation is strongly northern and eastern.

Species of southern range have gained a foothold in the border and drier portions and are recent arrivals.

The differences which exist in the anatomy and the vegetation habit of the various species do not adequately explain their distribution.

The results of the experiments indicate that the height of the water table is not an important factor in determining the distribution of the plants growing on this marsh meadow.

The meadow is well advanced toward a natural grass meadow with recognized greater hay values than the pure sedge meadows.

The utilization and improvement of this class of meadow lands is a question of considerable economic importance.

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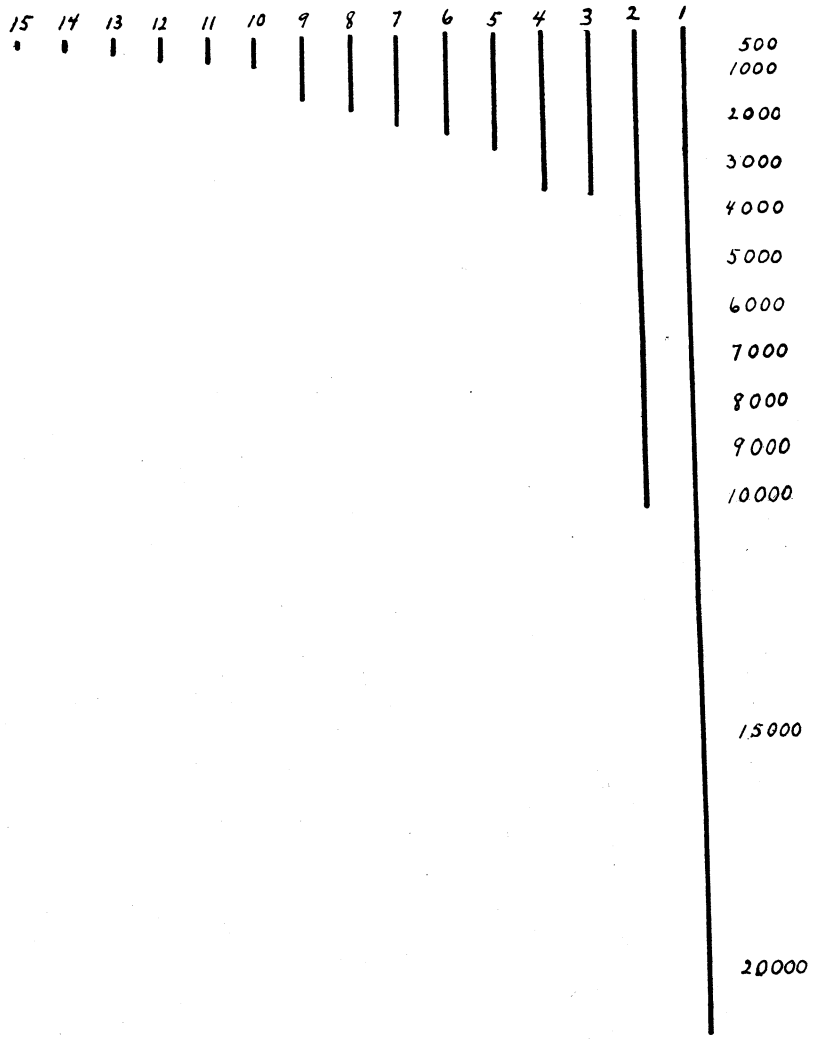
PLATE XVIII.



STOUT.

MARSH VEGETATION.

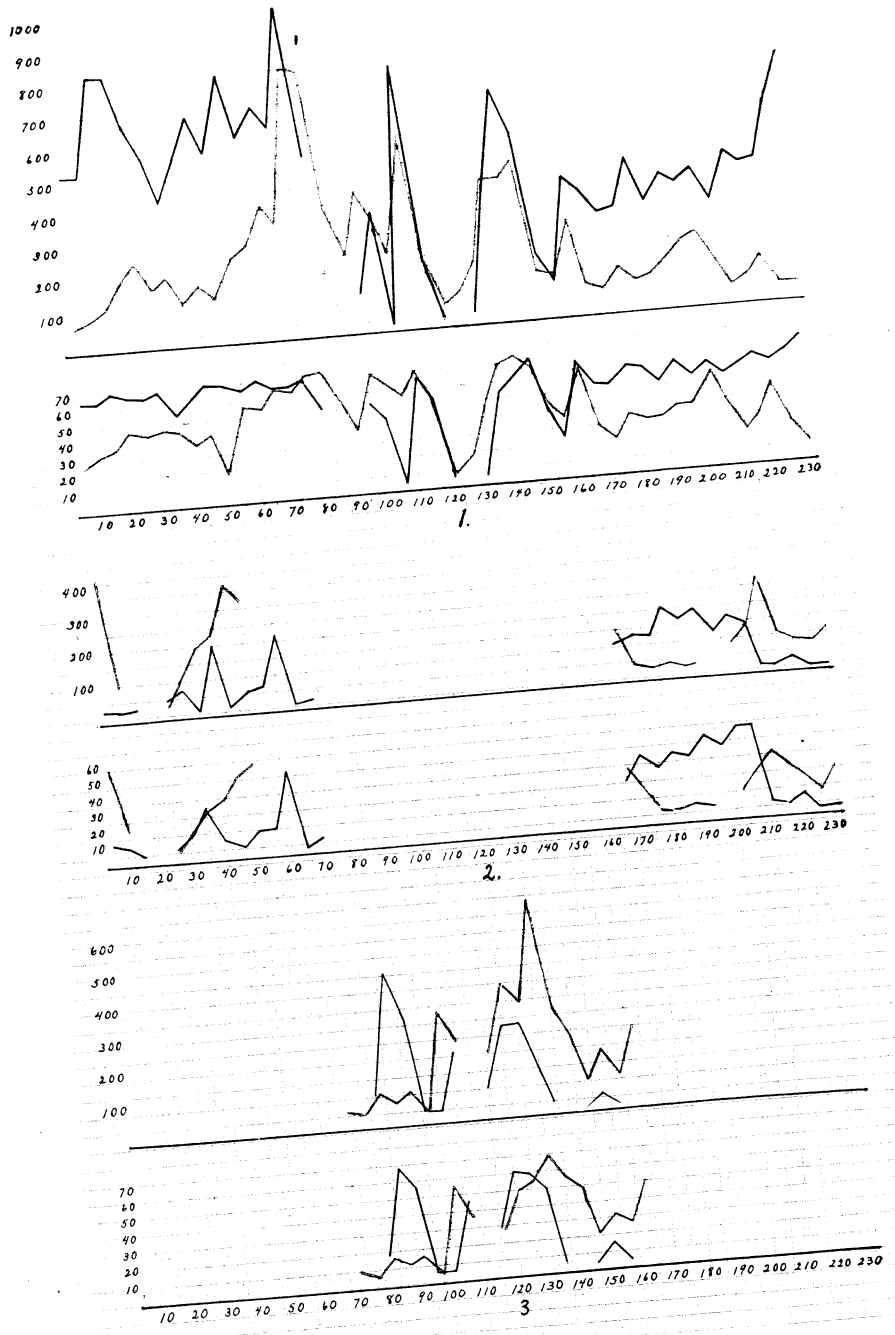
EXPLANATION OF PLATE XVIII.

The relative numerical proportions of the fifteen leading species as given in table 2 are here shown.

1. Carex stricta.
2. Calamagrostis canadensis.
3. Carex sterilis.
4. Carex Sartwellii.
5. Lycopus uniflorus.
6. Carex aquatilis.
7. Carex diandra ramosa.
8. Glyceria nervata.
9. Carx filiformis.
10. Agrostis alba.
11. Carex Bebbii.
12. Poa pratensis.
13. Scutellaria galericulata.
14. Audropogon furcatus.
15. Viola cucullata.

PLATE XIX.

TRANS. WIS. ACAD., VOL. XVII, PART I.



STOUT.

MARSH VEGETATION.

EXPLANATION OF PLATE XIX.

These plots show the distribution of the principal species. The upper plot of each set shows the numerical abundance and the lower one shows the corresponding frequency. In the former the value of each ordinate is 50 in the latter it is 10. The abcissa of both have the value of 50 feet. The highest frequency possible for a fifty foot segment, estimated as previously explained, is 75.

No. 1. Red is for Calamagrostis canadensis.
Black is for Carex stricta.

No. 2. Red gives the total combined values of Agrostis alba, Poa pratensis, audropogon fuscatus and Glyceria nervata.
Black is for Lycopus uniflorus.

No. 3. Red is for Carex Sartwellii.
Black is for Carex aquatilis.

PLATE XX.

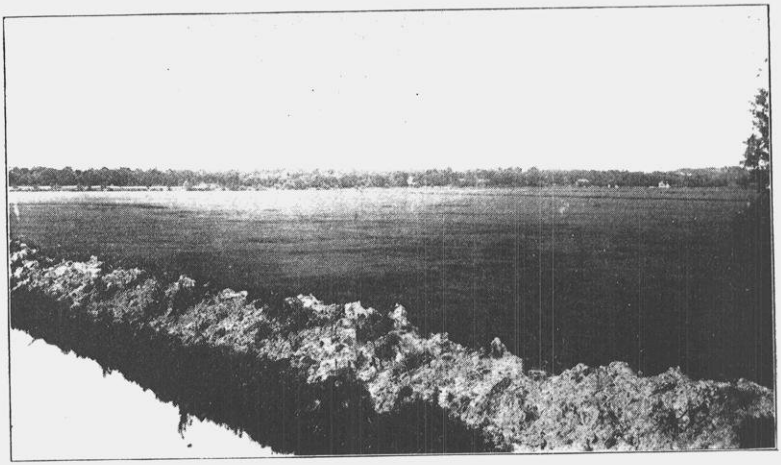


FIG. 1

FIG. 2

STOUT.

MARSH VEGETATION.

EXPLANATION OF PLATE XX.

Fig. 1. View of the marsh looking toward the east. The transect studied passes from south to north almost in a line with two half mile posts on the race track shown in the right center of this view. Photo was taken in August when the vegetation was fully developed.

Fig. 2. View looking northward from the 1120th foot of the transect. This shows a typical growth of Carex aquatilis and Carex Sartwellii. The measuring rod is here shown in place.

PLATE XXI.

FIG. 1

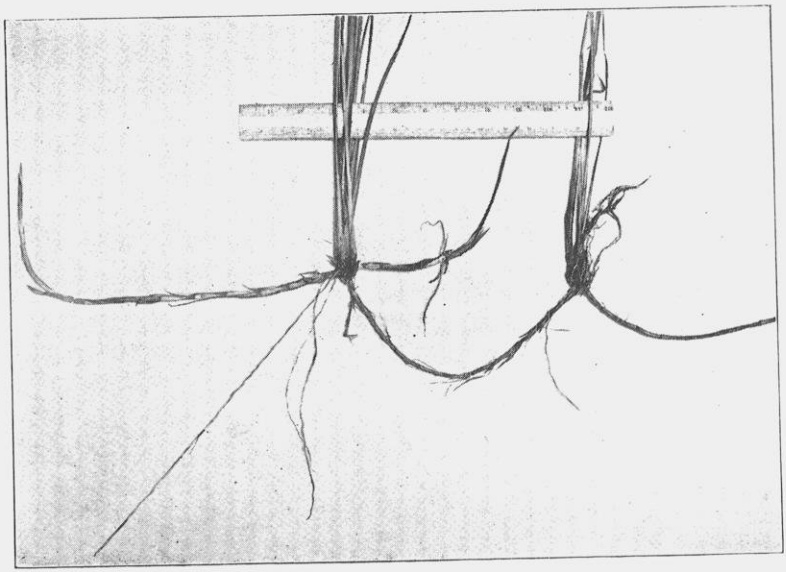


FIG. 2

STOUT.

MARSH VEGETATION.

EXPLANATION OF PLATE XXI.

Fig. 1. View looking south along the transect from the 1100 foot station. Taken in the midst of the Calamagrostis caricetum.

Fig. 2. A portion of a plant of Carex aquatilis as it grows in the soil. The view shows the rhizomes and their method of growth and the two kinds of roots which are produced.

PLATE XXII.

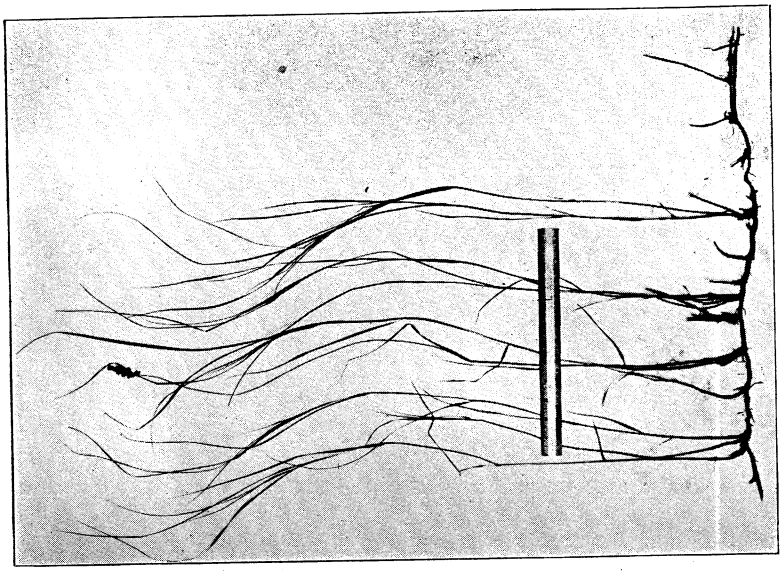


FIG. 2

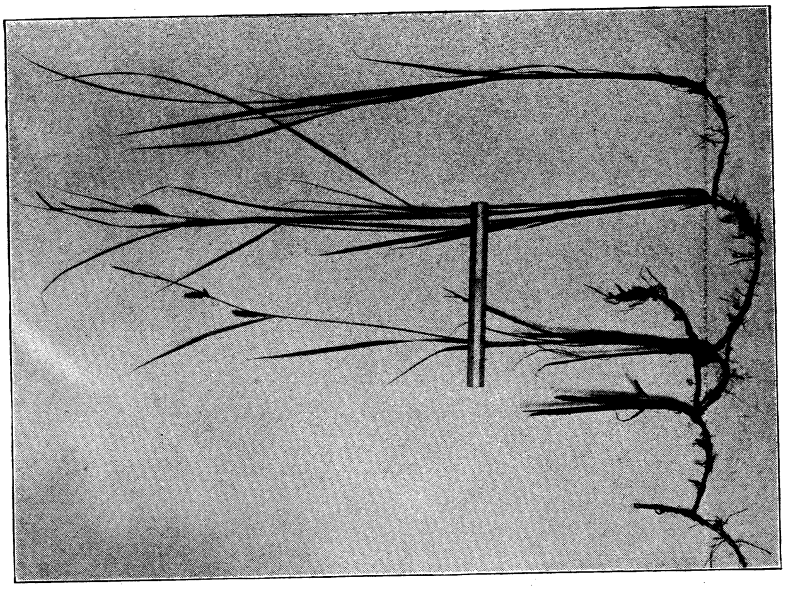


FIG. 1

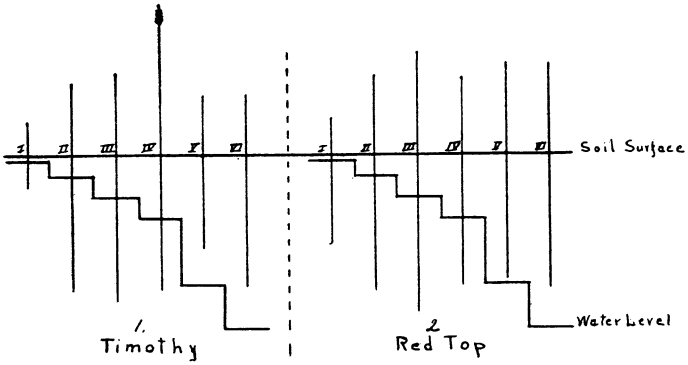
STOUT.

MARSH VEGETATION.

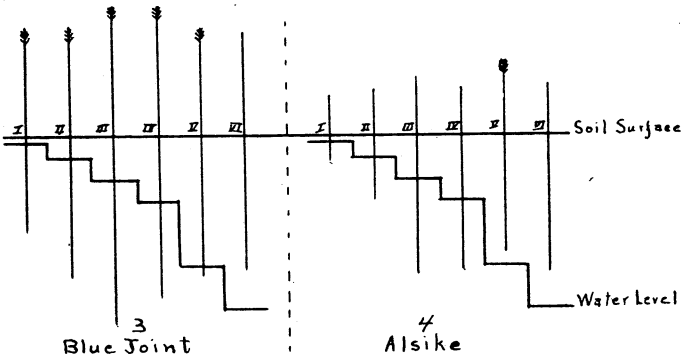
EXPLANATION OF PLATE XXII.

Fig. 1. Portion of a plant of Carex riparia.
Fig. 2. Plant of Carex Sartwellii.

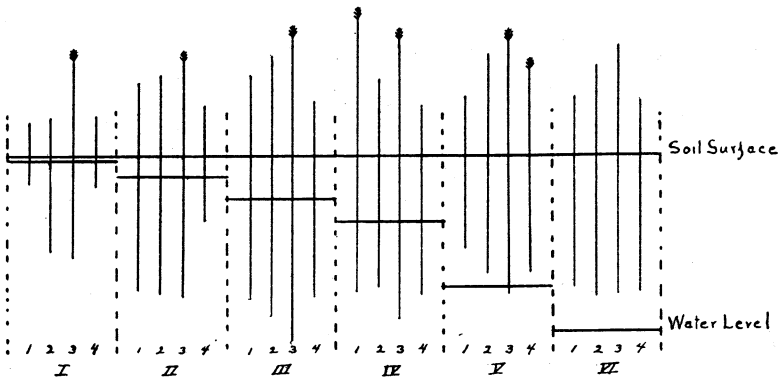
PLATE XXIII.


Soil Surface
Water Level
1.
Timothy
2
Red Top

1.


Soil Surface
Water Level
3
Blue Joint
4
Alsike

2


Soil Surface
Water Level
I
II
III
IV
V
VI

3.

EXPLANATION OF PLATE XXIII.

The results of the cylinder experiments are here graphically shown. The depths of the root growth and the heights of the shoot development are drawn to a scale with reference to the surface of the soil and to the water level. In plots 1 and 2 the results for each species are grouped together. In plot 3 the relative development of the four species in each cylinder is shown in succession.

SPECIES OF PHOLIOTA OF THE REGION OF THE GREAT LAKES

EDWARD T. HARPER.

The genus Pholiota includes plants with rusty or ochraceous spores, a well developed annulus, adnate or adnexed lamellae and a fleshy stem. These features are not absolutely distinctive of all the species for the color of the spores runs into the purple brown of Stropharia and the veil is sometimes as scanty as the fibers of a Flammula or clings to the margin of the pileus as in the genus Hypholoma.

Individuals in the same species often vary greatly and some different species are connected by numerous forms. As far as possible we have chosen characteristic plants for the photographs. Striking divergences from published descriptions have been noted but we have not made any new species when our plants failed to agree in all minor points with the descriptions of the species to which we have referred them.

We have endeavored to put together those species most alike in habit, structure and general appearance, arranging them under a series of types. The system of Fries has been followed. His three main divisions: plants growing on the ground, on wood, and among mosses are convenient though by following them some species like Pholiota terrigena and Pholiota angustipes are thrown out of their true relationship and the two similar types represented by Pholiota togularis and Pholiota marginata are widely separated. We have neglected the divisions based on the shade of color of the spores entirely as it is impossible to put similar plants together with that arrangement.

The genus includes plants of very diverse habits since it is based on only two characters, spore color and annulus. The most typical are the bright colored clusters of the squarrose and squamose group growing on the trunks of trees. They have the characteristic universal veil composed of matted fibers which tear apart as the plant expands leaving the stem annulate and the margin of the pileus appendiculate. Large plants growing on the ground of the type of Pholiota caperata can scarcely be distinguished from Stropharia. Others of the praecox-dura type are very similar to the appendiculate forms of Hypholoma and some of the species with smooth caps and scanty collars might almost as properly be placed in the genus Flammula. But all divisions are more or less artificial and spore color and annulus are perhaps as well marked characters as can be found on which to base a genus.

The species of Pholiota which grow on the trunks of trees are important from an economic point of view for they are quite as destructive to timber as some of the polypores. The mycelium grows for a long time in the wood before it is prepared to burst forth under favorable weather conditions into the handsome gold and yellow clusters which adorn the trunks of decaying trees in late summer and autumn.

Stevenson remarks that "none of the species of Pholiota can be commended as edible" but none of them are known to be poisonous or harmful. Some of them like Pholiota caperata are very delicious. No one need be afraid to try them.

We have not attempted to make an artificial key to the species but we think that the synopsis together with the pictures will enable anyone to recognize easily the plants we have had in hand. We have added under each type notes on the related species which are reported from the United States as far as they have come to our notice. These notes have been compiled from published descriptions. It is probable that some of the species reported, such as those described by Montagne from dried plants sent to him from Ohio by Sullivant, will never be identified.

Except in the cases noted the illustrations are natural size and give the average for the species. We have given few meas-

urements as it is difficult to fix the limits. There are few species in which very large and very small individuals do not occur.

The plants have been collected and photographed at different places in Michigan, Wisconsin and Illinois during the past four years. The district is quite rich in these plants and there is no reason why many of the species listed in the notes may not also be discovered here as well as a number of others that occur in Europe.

Synopsis of the Species.

A. Growing on the ground (Terrigenae).

1. Large plants with a thick persistent annulus. Type of Pholiota caperata.

Pholiota caperata (Pers.) p. 476, pl. XXIV.

Pholiota ..... p. 476, pl. XXV.

Related species: Pholiota johnsoniana, Pk.

2. Large plants with a membranous annulus.

Pholiota howeana, Pk. p. 477, pl. XXVI.

Related species: Pholiota ventricosa, Earle.

3. Medium sized plants with a membranous annulus. Type of Pholiota praecox and Pholiota dura.

Pholiota praecox (Pers.) p. 478, pls. XXVII and XXVIII, A, B.

Pholiota vermiflua, Pk. p. 478, pl. XXVIII, C–F.

Pholiota temnophylla, Pk. p. 479, pl. XXXIII, A.

Pholiota dura, Bolt. p. 480, pl. XXIX.

Related species: Pholiota mollicula, Bann. and Pk., Pholiota duroides, Pk.

4. Medium sized plants with a membranous annulus and viscid pileus. Type of Pholiota erebia.

Pholiota erebia, Fr. p. 481, pl. XXX.

Pholiota ombrophila, Fr. p. 482, pl. XXXI.

Related species: Pholiota aggericola, Pk. and Pholiota aggericola var. retirugis, Pk.

5. Small plants with a membranous annulus. Type of Pholiota togularis.

Pholiota togularis (Bull.) p. 482, pl. XXXII.

Related species: Pholiota togularis var. filaris, Fr., Pholiota blattaria, Fr., Pholiota rugosa, Pk., Pholiota anomala, Pk., Pholiota sabulosa, Pk.

6. Plants with a universal fibrous veil which leaves a fibrous annulus on the stem when the pileus expands, usually caespitose.

Pholiota terrigena, Fr. p. 484, pl. XXXIII, B.

Pholiota augustipes, Pk. p. 484, pl. XXXIV.

Pholiota speciosa, Clements and Pholiota rubecula, Bann & Pk. belong in the division Terrigenae.

B. Growing on Wood (Truncigenae).

I. Pileus and stem covered with squarrose or squamose scales (Squarrosae or Squamosae).

7. Type of Pholiota squarrosa.

Pholiota squarrosa, Muell. p. 485, pl. XXXV, A.

Pholiota squarrosa, var. verruculosa, Lasch. p. 486, pl. XXXV, B.

Pholiota squarrosoides, Pk. p. 486, pls. XXXVI and XXXVII.

Related species: Pholiota squarrosoides var. faginea, Pk., Pholiota subsquarrosa, Fr., Pholiota dactyliota, B. & M.

8. Type of Pholiota aurivella.

Pholiota aurivella, Batsch. p. 487, pls. XXXVIII, XXXIX.

Pholiota adiposa, Fr. p. 487, pl. XL.

Related species: Pholiota limonella, Pk., Pholiota villosa, Fr.

9. Type of Pholiota flammans.

Pholiota flammans, Fr. p. 488, pl. XLI, C.

Pholiota tuberculosa, Fr. p. 489, pl. XLI, A, B.

Related species: Pholiota curvipes, Fr., Pholiota hormomorpha, Mont.

10. Type of Pholiota albo-crenulata.
Pholiota albo-crenulata, Pk. p. 490, pls. XLII, XLIII.
Related species: Pholiota fusca, Quel. (Europe).

11. Type of Pholiota spectabilis.
Pholiota spectabilis, Fr. p. 490, pl. XLIV.
Related species: Pholiota aurea, Matt.

12. Type of Pholiota comosa.
Pholiota comosa, Fr. p. 491, pl. XLV.
Related species: Pholiota destruens, Broud.

13. Type of Pholiota heteroclita.
Pholiota heteroclita, Fr. p. 492, pls. XLVI, XLVII.

14. Type of Pholiota luteofolia.
Pholiota luteofolia, Pk. p. 492, pl. XLVIII.
Pholiota fulvo-squamosa, Pk. belongs to the section Squamosae.

II. Pileus nearly naked, sometimes rimose-rivulose (Aegeritinae).

15. Type of Pholiota aegerita.
Pholiota aegerita, Brigant. p. 493, pl. XLIX.

16. Type of Pholiota lutea.
Pholiota lutea, Pk. p. 493, pl. L.
Related species: Flammula alnicola, Fr.
Pholiota capistrata, Cke. Pholiota radicosa, Bull., Pholiota ornella, Pk. and Pholiota aeruginosa, Pk. belong to the section Aegeritinae.

III. Small plants with scaly or bristly pileus and stem.

17. Type of Pholiota muricata.
Pholiota erinaceëlla, Pk. p. 495, pl. LI.
Pholiota muricata, Fr. p. 495, pls. LII and LIII.

IV. Small plants with an hygrophanus pileus and membranous annulus (Hygrophanae).

18. Type of Pholiota marginata.

Pholiota marginata, Batsch p. 496, pls. LIV and LV.

Related species: Pholiota marginella, Pk., Pholiota unicolor, Fl. Dan., Pholiota mutabilis, Schaeff., Pholiota autumnalis, Pk., Pholiota discolor, Pk.

19. Type of Pholiota confragosa.

Pholiota confragosa, Fr. p. 497, pl. XLI, D. E.

Pholiota acericola, Pk. and Pholiota ceracina, Pk. belong to the section Hygrophanae.

C. Very small plants growing among mosses.

Pholiota mycenoides, Fr., Pholiota pumila, Fr., Pholiota rufidula, Kalchb. and Pholiota minima, Pk. are reported from this country.

A. Growing on the ground.

I. Large plants with a thick persistent annulus. Type of Pholiota caperata.

Pholiota caperata, (Pers.). Pl. XXIV.

WRINKLED PHOLIOTA.

A fine large species growing on the ground in woods and open places and mossy swamps, scattered or gregarious, frequent in this country and Europe. The specimens photographed were collected at Neebish, Mich., in September. They show the average size of the plants but the variation is great.

PILEUS firm and fleshy, small for the size of the stem, ovate when young becoming broadly bell shaped and expanded, obtuse, glabrous, yellow or alutaceous, usually covered with white flocci especially when young. The fibers wash off in wet weather and the pileus becomes somewhat soggy. In the plants I have seen the pileus is deeply striate. FLESH whitish, thin toward the margin, mild. LAMELLAE moderately close, adnate or broadly notched, more or less uneven on the edge, whitish becoming rusty with spores. STEM solid or stuffed, equal, white, glabrous or floccose, remains of the universal veil sometimes suggesting a volva at the base. ANNULUS thick and white, near the middle of the stem. SPORES elliptic, 7—8x13—14μ.

Pholiota ..... Pl. XXV.

Two collections of these plants were made in Sept., 1910, one at Blue Mounds and the other at Devil's Lake, Wis. They grew on the ground in thin woods.

They differ from Pholiota caperata in the shorter, floccose stems, the small spores, the squamose pileus and the striate annulus. The annulus is exactly like that of Stropharia bilamellata, Pk., and Dr. Peck to whom photographs and descriptions were submitted considered that the plants might belong to that species but recent collections of what seems to be true Stropharia bilamellata make the reference doubtful; the spores are very small and rusty brown, not purple brown, and the pile-

us is squamose. Striations or ridges on the annulus have been noted in Pholiota aurea, Pholiota rugosa, Pholiota togularis, var. filaris and in Stropharia coronilla. The ridges in our plants are very marked.

PILEUS fleshy, convex becoming plane, obtuse, even, slightly viscid when moist, smooth or squamose with innate darker colored fibers, yellowish. FLESH white. LAMELLAE close, adnate or slightly notched, becoming dark ferruginous brown with white eroded edges. STEM short, enlarged below, white floccose, solid becoming stuffed or hollow. ANNULUS thick with deep radiating ridges on the upper surface. SPORES ferruginous brown, elliptic, 3—4x5—6 μ.

The plant photographed is a very large specimen.

Note. Pholiota johnsoniana, Pk., is another species of this type. It is somewhat smaller than Pholiota caperata, has a similar thick annulus and even white stem. It was described and figured by Peck, N. Y. State Mus. Rep't 23 p. 98 and Pl. III. It is also described and photographed by Atkinson, Mushrooms, p. 153. Reported from Michigan by Kauffman.

2. Large plants with a membranous annulus.

Pholiota howeana, Pk. (?).

The plant photographed was found growing on the ground in open damp woods at Glencoe Ill., in June.

PILEUS heavy, fleshy, convex to plane or depressed when old, smooth, moist, even, dirty whitish or yellowish becoming dark colored. FLESH thick and white. LAMELLAE broad, ventricose, adnate, whitish becoming rusty brown with spores. STEM white. much thickened towards the base and fusiform rooted, slightly enlarging towards the apex, scaly and shreddy below the collar. ANNULUS broad, membranous, entire. Spores 4—5x8—9 μ.

The plant is close to Pholiota howeana, Pk., N. Y. State Mus. Rep't 26, pp. 59–60 (Stropharia howeana) and Mus. Bull. 122 p. 147.

Pholiota ventricosa, Earle, Bull. N. Y. Bot. Garden 1902 p. 341, is another species based on plants very similar to ours.

The plants grow in pine woods at Leland Stanford University, California. The habit, size and shape of the stem are very similar and the spore measurements exactly the same as in our plants but the colors are darker.

3. Medium sized plants with a membranous annulus. Type of Pholiota praecox and Pholiota dura.

Pholiota praecox, (Pers.). Pls. XXVII and XXVIII, A, B.

EARLY PHOLIOTA.

The early Pholiota is quite common on manured lawns and in grassy places during the spring and summer. The plants photographed were collected near Chicago in June.

PILEUS smooth, white with more or less yellowish or tan color, especially in the center, usually convex but sometimes umbonate. LAMELLAE broadly sinuate adnexed, slightly ventricose, whitish becoming rusty or fuscous brown. STEM white, smooth, more or less striate, even or slightly tapering downward, straight or flexuous. ANNULUS thin, membranous, separating from the pileus or from the stem. Spores rusty 6—8x10—12 μ.

The species is variable, Peck reports:

Var. sylvestris. Pileus with a brown center. Growing in woods. Var. minor. Small, with the veil separating from the stem and remaining as an appendiculate margin on the pileus.

Pholiota vermiflua, Pk. Pl. XXVIII, C—F.

WORMY PHOLIOTA.

The species is described in N. Y. State Mus. Rep't 31, p. 34 and figured in Mus. Bull. 75, pl. 86. It is closely related to Pholiota praecox but somewhat larger, cap whiter, often cracking into areas, stem striate above the annulus, lamellae darker brown and spores slightly larger. Its habitat is the same as that of Pholiota praecox but it occurs later in the season. Our plants were collected in the mulching by a fruit tree late in September. The cap was almost white and the lamellae darker brown than in Pholiota praecox. The spores were 8x12—13 μ.

The pilei were only slightly cracked. The veil has almost disappeared from the plant figured. Peck's description of the plant is as follows:

PILEUS convex or nearly plane, glabrous or sometimes floccose on the margin, commonly rimose areolate, especially in the center, white, sometimes slightly tinged with yellow. FLESH white. LAMELLAE close, adnexed, white, becoming ferruginous brown, generally minutely eroded on the edge. STEM equal, hollow, striate at the top, white, the white annulus more or less floccose on the lower surface, lacerated, often evanescent. SPORES 8x12—13 μ.

Pileus 2–4 inches broad; Stem 2–3 inches long, 3–5 lines thick.

Pholiota temnophylla, Pk. Pl. XXXIII, A.

CUT-GILLED PHOLIOTA.

The species is described in N. Y. State Mus. Rep't 23, p. 90 and in Mus. Bull. 122 p. 146.

PILEUS fleshy, hemispheric, becoming convex, smooth, ochraceous yellow. LAMELLAE very broad, adnexed, obliquely truncate at the inner extremity, brownish ferruginous. STEM equal, glabrous, hollow, white, the annulus well developed, membranous, white. SPORES brownish ferruginous, broadly elliptic 7—9x10—12 μ.

Pileus 1–2 inches broad, Stem 1–4 inches long, 2–4 lines thick. Grassy ground by roadsides in June. The plants resemble Naucoria semiorbicularis and Pholiota praecox.

Peck reports a single collection and Kauffman has found the species in Michigan. The plants photographed grew on the ground in grassy woods at Glencoe, Ills., in June. The gills of plants which were collected on Mackinac Island are even more typical of Dr. Peck's species, but the spores in both collections are narrower than in the type 6—7x10 μ. The identification has been confirmed by Dr. Peck.

Pholiota dura, (Bolt.). Pl. XXIX.

HARD PHOLIOTA.

Pholiota dura is very similar to Pholiota praecox, but the plants are heavier with a shorter, thicker, more irregular stem and the pileus tends to crack into areas. The two are found in similar localities, though Pholiota dura prefers gardens and fields, Pholiota praecox grassy places.

The plants pictured were found in a garden bed in June. They are mature and the pilei are well expanded. M. E. Hard, Mushrooms Edible and Otherwise p. 259 gives a good photograph of younger plants showing the incurved pileus with a rimose areolate surface. In our plants there are striations on the stem above the annulus like those on the stem of Pholiota vermiflua.

PILEUS firm, fleshy, smooth or cracked into areas, margin incurved when young becoming convex or expanded, obtuse, even on the margin, yellowish tawny or tan color, becoming darker. FLESH firm, brittle, white, mild. LAMELLAE ventricose, adnexed, with a slight sinus and a striate decurrent tooth, whitish, becoming the color of the spores. STEM stuffed or solid, even, tapering downward, more or less ventricose, fibrous, widening to the pileus, whitish. ANNULUS thin and fragile, entire or torn. SPORES rusty brown, 5—6x8—9 μ.

Note. **The two following species described by Peck appear to belong to the praecox-dura type.**

Pholiota duroides, Pk. **N. Y. State Mus. Bull. 122 p. 148. It is separated from Pholiota dura by its more ochraceous pileus, softer substance and smaller spores, 4—5x6—7μ.**

Pholiota mollicula **Bann. and Pk., N. Y. State Mus. Rep't 44 p. 70, is a plant of the same character. It has a whitish pileus with the disk yellowish, gills whitish becoming cinnamon, stem white, stuffed or hollow, annulus large and white and spores rusty 5x8μ. Growing in woods at the roots of trees.**

This large number of species shows the great variability of the praecox-dura type of Pholiota. More forms might be distinguished for plants are often collected that do not agree ex-

actly with any of those described. A careful study of the group would probably show that all the species are connected by intermediate forms. A number of European botanists have doubted whether even Pholiota praecox and Pholiota dura should be considered specifically distinct.

4. Medium sized plants with a membranous annulus and viscid pileus. Type of Pholiota erebia.

Pholiota erebia, Fr. Pl. XXX.

DARK PHOLIOTA.

The plants from which the photographs were made were collected at Blue Mounds, Wis., in September. They grew scattered or in small clusters on the ground in damp woods. More mature plants have been found at Neebish, Mich. Some of them had the pileus slightly umbonate. The margin of the pileus was distinctly striate and the annulus more remote than in the plants from Blue Mounds.

PILEUS fleshy in the center, thin on the margin, convex, becoming plane or somewhat umbonate, viscid, smooth or rugose wrinkled, hygrophanous, striate on the margin when mature, brown or blackish when moist, lighter when dry, fully dried specimens are clay color. LAMELLAE adnate with a tooth, pallid or grayish, becoming rusty. STEM equal or slightly tapering upward, stuffed or hollow, striate with innate fibers and squamulose especially towards the base, stems often cohering at the base, whitish above the annulus, darker below. ANNULUS near the top of the stem, becoming distant, membranous, reflexed, sulcate, white. SPORES 6x12—14 μ.

Massee remarks of the European plant that the pileus is sometimes more or less umbonate and Stevenson says that many of the stems cohere at the base where they are squamulose, also that the pileus is often wrinkled. Our plants show these characters.

Note. The plants agree with the description of Pholiota aggericola, Pk. N. Y. State Mus. Bull. 122 p. 146 except in the sometimes umbonate pileus, the hollow stem and the slightly longer spores. 12—14μ

instead of 10—12μ. The hollow stem and longer spores are given as characters of Pholiota indecens, Pk., N. Y. State Mus. Rep't 30, p. 40, which Peck has decided is not distinct from Pholiota aggericola, Mus. Bull. 122, p. 146. Hence it is probable that both Pholiota indecens and Pholiota aggericola are the same as Pholiota erebia, Fr. Pholiota aggericola retirugis is the form with a wrinkled pileus and is further proof of the identity of the species.

Glatfelter reports Pholiota aggericola from St. Louis with spores 5—6x12—15μ. Trans. Acad. Sci. St. Louis. 1906.

Pholiota ombrophila, Fr. Pl. XXXI.

RAIN-LOVING PHOLIOTA.

The plants illustrated were collected near brush piles in a grassy pasture at Geneseo, Ills., in June. Others have been found at Lake Geneva, Wis., and elsewhere, always about brush piles and in very wet weather. The plants photographed were somewhat above the average size. They agree well with the description of Pholiota ombrophila but the pilei are not so dark colored as in the figures given by Cooke, illust. pl. 359 and Fries Icones 103. The latter is var. brunneola. The color of the pileus is described as "pale ferruginous."

PILEUS fleshy, convex to expanded, smooth, slightly viscid, hygrophanous, irregular or wavy on the margin, striatulate, pale ferruginous. FLESH whitish. LAMELLAE sinuate attached with a decurrent tooth, narrowing outward from the stem, whitish becoming rusty. STEM stuffed or hollow, whitish, flexuous, somewhat fibrous striate. ANNULUS membranous, broad, entire, white. SPORES ferruginous, 5—6x8—9 μ. (Stevenson gives 4x8 μ and Saccardo 6—7x13—14 μ.)

5. Small plants with a membranous annulus. Type of Pholiota togularis.

Pholiota togularis, (Bull.) Pl. XXXII.

LITTLE CLOAK PHOLIOTA.

Pholiota togularis is a common species growing on the ground or attached to sticks in open woods and pastures. We have col-

lected it at Madison, Wis., Geneseo, Ills., and elsewhere. The photographs in pl. XXXII, A are from the Madison plants. They are old with the pileus depressed and the annulus almost gone and are not very satisfactory. The plants in pl. XXXII, B were more hygrophanous, with the margin of the pileus slightly striate and the gills bent in the middle. They were quite wrinkled when dry and suggest a form of Pholiota rugosa, Pk. We have no good photograph of Pholiota togularis which is a pretty little plant with a broad membranous annulus, the "little cloak" which suggested the name.

PILEUS thin, soft, convex to plane or depressed, smooth, even on the margin, somewhat hygrophanous, pale ochraceous, almost white when dry. LAMELLAE adnate or toothed-decurrent, ventricose, yellowish-white becoming ochraceous. FLESH thin, soft, yellowish. STEM hollow, flexuous, somewhat fibrillose, colored like the pileus, darker below. ANNULUS membranous, evanescent, near the middle of the stem. SPORES ochraceous, 5x8 μ.

Note. Plants of the Pholiota togularis type form a very variable group. A number of species and varieties have been reported from this country. They are all small plants growing on the ground or attached to sticks and very closely related to each other.

Pholiota togularis, var. filaris, Fr. is reported by Peck and raised to the rank of a species. Mus. Bull. 122, p. 144. It is characterised by its small size slightly striate pileus and very thin stem. Figured in Fries Icon. pl. 104.

Pholiota blattaria Fr. is reported in Farlow's Index. It is like a slender form of Pholiota togularis but has the pileus striate on the margin and the gills free.

Pholiota rugosa, Pk., N. Y. State Mus. Rep't 50 p. 102 and Mus. Bull. 122, p. 144, is a more hygrophanous plant with the pileus slightly striate on the margin, the annulus with striae on the upper surface and the pileus rugose wrinkled when dry. It is reported from Michigan by Kauffman.

Pholiota anomala, Pk., Torr. Bull. 22, p. 202, was described from plants growing on sticks and leaves lying on the ground at Pasadena, California. They are about the size of Pholiota togularis, brown, drying cream color, with adnate lamellae and a fugaceous annulus. The stem is hollow with transverse partitions, the internodes stuffed with a cottony substance. Pholiota dissimulans, B. & Br. has such nodes in the stem, Cooke, Illust. pl. 371.

Pholiota sabulosa, Pk., Torr. Bull. 23, p. 414, is another similar plant which grew on sandy soil in Alabama. It is the same size as the others, yellowish brown becoming pale tawny when dry, with a concolorous hollow stem, and a slight annulus, but there were rusty brown scales on the pileus.

6. Plants with an evident universal veil and ragged fibrous annulus. Growing on the ground, scattered or caespitose.

Pholiota terrigena and Pholiota angustipes are placed here because they grow on the ground. The former belongs to the Squarrosae and the latter to the Squamosae.

Pholiota terrigena, Fr. Pl. XXXIII, B.

The plants were found at Devil's Lake, Wisconsin, September. They grew in clusters on the ground in open woods. The photograph shows a bunch of young plants. The caps expand and become almost plane.

PILEUS convex, margin incurved so that in young plants the cap is lens shaped, becoming expanded and plane, dry, covered with a coat of silky matted fibers, more or less torn and fibrillose scaly especially on the margin, dull yellow or tawny, scattered over the surface are tawny, verrucose, easily separable scales like those on the stem. FLESH yellowish, LAMELLAE becoming rusty with an olivaceous tint. STEM stuffed or hollow, squarrose with tawny, verrucose scales in a web of white fibers, silky white above the annulus. ANNULUS the torn margin of the universal veil part of which adheres to the pileus. SPORES ochracous 4x5—6 μ.

Our plant agrees with the illustration in Fries, Icones, 103. Cooke's plate 349 is too bright yellow and does not well represent our forms.

Pholiota angustipes, Pk. Pl. XXXIV.

NARROW STEM PHOLIOTA.

The plants grew in clusters on the ground in a place where stumps had been removed in a pasture near Madison, Wis., in September. The average size is shown by the photographs. Our

plants agree exactly with the description of Pholiota angustipes except that the spores are a little smaller, 4x6—7 μ.

PILEUS fleshy, hemispheric, becoming convex or nearly plane, slightly viscid when moist, squamulose with minute, dot like appressed scales, brown or grayish brown becoming alutaceous brown or subalutaceous, FLESH whitish, taste unpleasant. LAMELLAE thin, close, sinuate, whitish or creamy yellow, becoming tawny brown. STEM equal or tapering downward, flexuous, stuffed or hollow, squamose, whitish or cinereous, SPORES naviculoid, 4—5x7—8 μ.

Note. Two species of Pholiota which grow on the ground but do not appear to be very closely related to any of the above types have been reported from the United States.

Pholiota speciosa, Clements, University of Nebr. Bot. Sur. 1893, II, p. 41, is said by the author to resemble Pholiota gibberosa, Fr. It is about two inches high and broad, has a sordid white pileus and white stem, smoky gills and umber spores 3.5x5μ.

Pholiota rubecula, Bann. & Pk. N. Y. State Mus. Rep't 44, p. 70, is not fully described.

B. Growing on wood.

I. Pileus and stem covered with squarrose or squamose scales.

The plants are clothed with a universal fibrous veil which forms squarrose or squamose tufts of fibers on the pileus and stem. When the margin of the pileus separates from the stem the veil tears apart leaving a floccose ring on the stem and the margin of the pileus ragged.

7. Type of Pholiota squarrosa.

Pholiota squarrosa, Muell. Pl. XXXV, A.

SCALY PHOLIOTA.

One of the most common and best known of the species of Pholiota. It grows in dense clusters on standing trunks, stumps and logs in woods. It sometimes has a very disagreeable odor.

PILEUS fleshy, broadly conic or campanulate to convex, dry, background yellowish or tawny covered with darker tawny

squarrose scales. LAMELLAE adnate or slightly notched decurrent, whitish becoming ferruginous. STEM straight or flexuous, colored and adorned like the pileus, white furfuraceous above the annulus. SPORES elliptical 4x7—8 μ.

There are a number of varieties of the scaly Pholiota. Plate XXXV, A shows two plants nearly typical. The pileus was dry with ragged tawny scales on a paler tawny background and Spores 4x7 μ. The plants were collected at Blue Mounds, Wis., in October. Other collections have still darker caps but the spores are usually smaller.

Pholiota squarrosa, var. verruculosa, Lasch. Pl. XXXV, B.

The plants were collected at Frankfort, Mich., in August. The pilei were yellow with hard, sharp, verrucose, tawny scales.

Pholiota squarrosoides, Pk. Pls. XXXVI and XXXVII.

SHARP SCALE PHOLIOTA.

Pholiota squarrosoides is described and figured in N. Y. State Mus. Rep't 54, p. 183, pl. 73 and Pholiota squarrosa in Mus. Bull. 54 p. 971 and pl. 79. The former has the background of the pileus whitish and viscid instead of tawny and dry as in Pholiota squarrosa, it has sharp instead of flat scales and smaller spores, 3—4x5 μ instead of 4—5x7—8 μ. The plants illustrated in pls. XXXVI and XXXVII have these characteristics. They were collected on a well decayed log at Frankfort, Mich., in July. The log was covered with large handsome clusters. This form seems to be more common in our regions than Pholiota squarrosa but I have collected plants with white and tawny caps growing side by side. The color becomes darker with age and the viscidity depends much on the weather. Both spore measurements are reported by Stevenson, British Fungi I, p. 230.

Dr. Peck has distinguished and figured the form on beech logs, Pholiota squarrosoides faginea, Pk., N. Y. State Mus. Rep't 54, p. 183 and pl. 73. It is a smaller plant with more scattered scales. He finds Pholiota squarrosoides on maple logs and this form on beech.

Note. **Two more plants of the Pholiota squarrosa type are reported in this county.**

Pholiota subsquarrosa, Fr. McIlvaine. One thousand American Fungi, p. 275. The plants have a viscid pileus and appressed scales. The gills are yellow when young. The species is figured by Fries Icones, 103.

Pholiota dactyliota, B. & Mont. is a little known species described from plants collected by Sullivant in Ohio. It is said to be so similar to Pholiota squarrosa as scarcely to need a description. The only differences are that the annulus is thick and persistent and the gills nearly free.

8. Type of Pholiota aurivella.

Pholiota aurivella, Batsch. Pls. XXXVIII and XXXIX.

GOLDEN FLEECE PHOLIOTA.

A very showy plant growing singly or in clusters of few individuals on trunks, stumps and logs. The illustrations are from plants found on a well decayed bass wood log at River Forest, Ills., in October, also collected in Colorado and elsewhere. Very similar to the following species as Cooke's Illustrations pls. 351 and 353 well show, but much more handsome. Stevenson remarks "Very beautiful. More refined in appearance than any of its allies."

PILEUS broadly convex, gibbous, splitting on the margin, slightly viscid when moist, smooth and almost glassy when dry, bright tawny yellow or orange, scattered over with tufts of dark tawny fibers, appressed with squarrose points. LAMELLAE ventricose, adnexed with a small sinus, whitish or yellowish becoming rusty brown with spores. STEM even, or somewhat fusiform, solid, curved to match the position of the plant, lighter yellow than the pileus, very smooth and polished above the annulus, shreddy and tawny scaly below. Annulus slight, formed by the torn margin of the veil. SPORES rusty brown. 4—6x8—9 μ.

Pholiota adiposa, Fr. Pl. XL.

FAT PHOLIOTA.

The Fat Pholiota is much more common than the preceding and forms large clusters on trunks, stumps and logs. The pho-

tograph is from part of a large cluster taken from the trunk of a maple tree at River Forest, Ills., in June. It is a much less trim plant than the Golden Fleece Pholiota and the colors are not nearly so bright. The two can easily be distinguished even from dried specimens.

PILEUS convex or expanded, broadly umbonate, dingy yellow with the scaly tufts of fibers brownish or blackish. Scales easily separable leaving the pileus smooth. FLESH thick, dull yellow. LAMELLAE slightly notched, dirty yellow becoming brown. STEM even or slightly thickened downward. Scaly below the annulus, furfuraceous above, yellowish with a tawny or brown base. ANNULUS the slight remains of the torn veil, soon disappearing. SPORES rusty brown 5x8 μ.

Note. Two species reported from this country are said to be closely related to Pholiota adiposa.

Pholiota limonella, Pk. N. Y. State Mus. Rep't 31, p. 33. It grows in clusters on beech trunks and resembles Pholiota adiposa. The plants are about the size of Pholiota flammans but the spores are twice as large as in that species, 5—6x8—9μ. They are lemon yellow with erect reddish brown scales on the pileus and stem. Morgan reports the plant from Ohio.

Pholiota villosa, Fr. is a rare species in Europe. The plants are about the size of Pholiota adiposa with tawny yellow, floccose, fibrillose pilei and stems. It is reported in Farlow's Index.

9. Type of Pholiota flammans.

Pholiota flammans, Fr. Pl. XLI, C.

YELLOW SCALE PHOLIOTA.

This beautiful little plant differs from the others of the section Squarrosae in having the scales lighter colored than the background. The pileus is deep yellow or tawny and the scales sulphur yellow. It grows singly or in tufts on stumps and trunks. The one photographed grew on a stump at Neebish, Mich., in September. It is a small plant, the pileus less than two inches broad.

PILEUS thin, fleshy, convex to plane, slightly umbonate, dry, yellow or tawny with paler yellow scales. FLESH yellowish.

LAMELLAE notched attached, yellowish becoming ferruginous. STEM straight or curved, stuffed or hollow, yellow and adorned like the pileus. ANNULUS near the top of the stem, ragged. SPORES rusty 3 x 5 μ.

The plants retain their color when dry. Ours became covered with a yellow powder like the pulverulence on some Boleti. Fries, Icon. 104, beautifully illustrates the plant. It is reported from Michigan by Kauffman.

Pholiota tuberculosa, Fr. Pl. XLI, A. B.

TUBERCULATE PHOLIOTA.

Pholiota tuberculosa is similar to Pholiota flammans but the scales are concolorous with the background or darker, and there is a beautiful round bulb at the base of the stem. The illustration in Fries, Icon. 104, represents our plants exactly. They were collected at Neebish, Mich., in September. The photograph is taken from a dried plant.

PILEUS fleshy, convex, obtuse, beautiful tawny yellow, with more or less squarrose, scattered tawny scales. STEM hollow, incurved, bulbous at the base, fibrillose scaly, colored like the pileus. LAMELLAE adnexed, yellow becoming rusty. ANNULUS the ragged upper margin of the scaly part of the stem. SPORES rusty ochraceous, inequilateral 3 x 5—6 μ. (Stevenson 4x7 μ, Sacc. Sylloge 4—5x8—10 μ.

Note. Pholiota hormomorpha, Mont., described from plants collected at Columbus, Ohio by Sullivant is said to be very similar to Pholiota tuberculosa. The stem is thickened at the apex as well as bulbous at the base and naked. Spores oblong.

Pholiota curvipes, Fr. is reported from this country by Peck, Hard and others. In Farlow's Index it is given as identical with Pholiota tuberculosa and according to Longyear it has been confused with Pholiota muricata in this country.

10. Type of Pholiota albo-crenulata.

Pholiota albo-crenulata, Pk. Pls. XLII and XLIII.

WHITE GRANULATED PHOLIOTA.

Single or two or three together on stumps and logs especially maple. The photographs are from plants found on a maple stump at Frankfort, Mich. The characteristic features of the plant are the dark brown color, easily recognized even in dried specimens and the white granules on the margin of the gills.

The species is very closely related to Pholiota fusca, Quel. and may prove to be identical with it. Plants in the Madison herbarium were so referred by Bresadola and the description of the gills of that species as "white granulate" on the edge is better for our plants than "white crenulate." But both the description and figure of Pholiota fusca show that it is strikingly mammillate and it is said to be caespitose. We have seen no American plants with these characteristics.

PILEUS, fleshy, convex or with a small umbo, viscid, yellowish brown with dark brown floccose scales which easily rub off. STEM slightly tapering upward, stuffed or hollow, covered up to the annulus with dark brown tufts of fibers on a light colored background. White furfuraceous above the annulus which has the form characteristic of this group. LAMELLAE with a peculiar appearance, those reaching the margin narrowing toward the stem and those attached to the stem narrowing toward the margin, edge eroded and beaded with white granules, grayish becoming rusty brown. Spores, rusty brown, 6—7x10—12μ.

The plant is reported from Michigan by Kauffman.

11. Type of Pholiota spectabilis.

Pholiota spectabilis, Fr. Pl. XLIV.

SHOWY PHOLIOTA.

The whole plant including the flesh is some shade of bright yellow or orange and retains its color when dry. The plants photographed were collected at Neebish, Mich., in September. They are young but show the characteristics of the plant well.

The thick matted veil covers the whole plant when young. It tears apart at the separation of the pileus from the stem and leaves the stem peronate and the margin of the pileus covered with bunches of fibers. The pileus is scaly but not squarrose.

PILEUS fleshy, compact, hemispherical, becoming nearly plane, dry, silky fibrillose, yellow to tawny orange. FLESH thick, pale yellow, bitter. LAMELLAE close, narrow, adnate or slightly decurrent, yellow becoming ferruginous. STEM ventricose or thickened below, solid, peronate, mealy above the annulus, fibrillose like the pileus below. SPORES elliptic, ochraceous, 5—6x8—9μ.

Fries, Icones 102, gives a good illustration of our plant.

Note. Pholiota aurea, Matt., which is the type of the genus and its most gorgeous species, is closely related to Pholiota spectabilis. It grows on the ground. The plant is reported from this country in Farlow's Index but we have never seen it.

12. Type of Pholiota comosa.

Pholiota comosa, Fr. Pl. XLV.

HAIRY PHOLIOTA.

A firm fleshy species growing on trunks and stumps of deciduous trees. The pictures are from plants collected at Frankfort, Mich., in August and at River Forest, Ill., in October.

PILEUS firm, convex, obtuse, viscid, covered with white hairy fibrous easily separable scales on a tawny ground. FLESH white. LAMELLAE broad, adnexed decurrent, white becoming argillaceous or reddish brown. STEM somewhat bulbous with an abrupt pointed root becoming long and curved, white fibrous striate with the characteristic slight annulus of the section. SPORES rusty brown 5—6x8—9μ.

Note. Pholiota destruens, Broud. is reported from Missouri by Glatfelter and dried specimens in the herbarium at Madison seem to be referable to this species. The pileus is yellowish white with a few floccose scales and a fibrillose margin. The stem is concolorous and thickened below. The lamellae are pallid becoming cinnamon.

13. Type of Pholiota heteroclita.

Pholiota heteroclita, Fr. Pls. XLVI and XLVII.

ECCENTRIC STEMMED PHOLIOTA.

A large, heavy, dull colored plant, often with an eccentric stem, growing on stumps and logs of deciduous trees. Our plants were deeply rooted in a crack on the top of a poplar stump at Frankfort, Michigan. They were fully mature. Hard, Mushrooms Edible and Otherwise, fig. 214, has published a photograph of young plants which shows the characteristic veil and annulus.

PILEUS whitish, covered with dirty yellow, or tawny fibrous scales, incurved when young, becoming convex and plane, margin incurved, often irregular and cracked or split. STEM often eccentric, solid, bulbous at the base, rooting below the bulb, whitish fibrous below the annulus which is near the top of the stem, mealy above. FLESH thick, white. LAMELLAE broad, rounded at the stem, pallid becoming ferruginous brown. SPORES rusty, 5—6x8—10μ.

14. Type of Pholiota luteofolia.

Pholiota luteofolia, Pk. Pl. XLVIII.

YELLOW GILLED PHOLIOTA.

We photographed some individual plants taken from a cluster which grew on a decayed log at River Forest, Ill., in June. The plants were fully mature and the pilei depressed showing the brilliant reddish yellow gills as the clusters stood erect on the top of the log attracting the attention at some distance.

Peck's description reads "PILEUS fleshy, firm, convex (ours were depressed and moist from the wet weather), dry, squamulose, fibrillose on the margin, pale red or yellowish. LAMELLAE broad, subdistant, sinuate, serrate on the edge, yellow becoming bright ferruginous. STEM firm, fibrillose, solid, often curved from its place of growth. ANNULUS slight, fugacious. SPORES bright ferruginous 4x7μ" (ours were 4—5x7—8μ).

Note. Pholiota fulvosquamosa, Pk., Torr. Bull. 30 pp. 95–96, belongs in the section Squamosae. The plants on which the species is founded were collected about the base of oak trees near the Agricultural College at Lansing, Michigan. The pileus is 6–12 cm broad, the stem is 5–8 cm. long, and 8–10 mm. thick. The pileus, the stem and the under side of the annulus are covered with tawny fibrillose scales. The lamellae are attached to a narrow collar, whitish becoming pinkish cinnamon.

II. Pileus naked, sometimes rimose rivulose.

15. Type of Pholiota aegerita.

Pholiota aegerita, Brigant. Pl. XLIX.

The plants photographed were not very satisfactory and the pictures are poor. They grew on a poplar trunk at Neebish, Mich. The dried specimens are characteristic. The pileus is inrolled, hard, cracked into tawny areas on a whitish background, smoother and whitish toward the margin. The stem tapers upward and is brownish at the base.

PILEUS fleshy, convex to plane, rivulose with tawny scales in the center, smoothish and white or pallid toward the margin, with slight greenish tints. LAMELLAE adnate toothed, pallid becoming reddish brick color. STEM equal or tapering upward, solid or stuffed, whitish with silky, brownish or reddish fibers. ANNULUS superior, fibrous. SPORES 5x7μ (Sacc. Sylloge, 5x8—9μ).

16. Type of Pholiota lutea.

Pholiota lutea, Pk. Pl. L.

YELLOW PHOLIOTA.

The plants referred to this species were very abundant and grew in large clusters on the trunks and roots of black birch at Spring Green and The Dells, Wis., during September and October, 1910. The mature pilei were broadly conical or campanulate, buff yellow, nearly smooth, wavy, somewhat scaly and striate on the edge. The stem was brown or ferruginous toward

the base and had a well defined annulus. The spores were 5x8—9μ. The plants are very closely related to Flammula alnicola and may belong to that species, but they differ very decidedly in the shape and color of the pileus and the evident annulus from the forms of Flammula alnicola collected in northern Michigan. The shape of the pileus and the dark base of the stems which are sometimes hollow do not agree with the description of Pholiota lutea. Glatfelter has reported Pholiota lutea from Missouri. Peck's description is as follows:

"Pileus fleshy, firm, convex, dry, slightly silky and sometimes minutely floccose squamulose in the center, buff yellow, often a little darker in the center, the thin incurved margin slightly surpassing the lamellae. FLESH pale yellow. ODOR pleasant. TASTE bitter. LAMELLAE thin, close, rounded behind, adnexed, pale yellow, becoming dark ferruginous. STEM firm, solid, thickened at the base, fibrillose, colored like the pileus. ANNULUS superior, slight, fugacious. SPORES ferruginous 5x8μ. Pileus 2–4 inches broad; stem 2–3 inches long, 3–5 lines thick. Decaying wood and trunks of trees in woods."

Note. The following species, reported from this country, appear to belong in this section.

Pholiota ornella, Pk. is a small plant found growing on decayed wood or sawdust. Pileus dark red when young fading to pink and then yellowish brown, appressed scaly, veil annulate appendiculate. Its history is given in N. Y. State Mus. Bul. 122, p. 151. It was first described as a Hypholoma.

Pholiota aeruginosa, Pk. is a plant with a greenish pileus and stem, less than two inches broad and one and one-half inches high. Distinguished from Stropharia aeruginosa by its solid stem, dry pileus and bright ferruginous spores. The type specimens were found by Dr. Herbst growing in clusters on oak railroad ties in Pennsylvania. N. Y. State Mus. Rep't 43, p. 81. The plant is also reported from Connecticut, White, and Michigan, Longyear.

Pholiota capistrata, Cke is reported in Farlow's Index. It is figured in Cooke, Illust. 364. A large subcaespitose plant with a viscid livid pileus, a subsquamulose stem and persistent annulus, growing on fragments of wood.

Pholiota radicosa, Bull. is also reported in Farlow's Index. It is a large plant with smooth pileus, squarrose scaly stem and a long root. It appears to grow on the ground though placed among the Truncigenae in Sylloge.

III. Small plants with scaly or bristly pileus and stem.

The plants in this division are squarrose or squamose, but they are small, grow on logs in woods and resemble those of the following section much more closely than the showy forms of the type of Pholiota squarrosa.

17. Type of Pholiota muricata.

Pholiota erinaceëlla, Pk. Pl. LI.

LITTLE BRISTLY PHOLIOTA.

The plant was described as Agaricus (Pholiota) detersibilis, Pk. in N. Y. State Mus. Rep't, 28, and the name was changed to Pholiota erinaceëla in Mus. Bull. 122, p. 152. The bristly pileus and stem is well shown in the photographs. The plants agree with the description exactly. They grew on logs in woods at Frankfort, Mich., in August. Peck's description reads:

"PILEUS thin, hemispheric or convex, dry, densely coated with small, erect, separable, pyramidal or spinelike scales, tawny brown. LAMELLAE broad, close, adnexed, pallid becoming cinnamon brown. STEM equal, stuffed or hollow, densely squamulose below the slight annulus, often curved, colored like the pileus. SPORES ferruginous, naviculoid 4–5x8–9μ. Pileus 6–12 lines broad, stem 6–12 lines long, 1 line thick."

Pholiota muricata, Fr. Pls. LII and LIII.

The plants pictured in Pl. LII were collected at River Forest, Ill., in June, those in Pl. LIII at Neebish, Mich., in September. The River Forest plants are slightly heavier, neater, and more squarrose than those found at Neebish but they seem to be the same species. All were tawny yellow with bunches of bright yellow mycelium at the base of the stems. The plants represented in Pl. LII, B, had long straggling stems due to their position emerging from a crack in the bark of the log.

PILEUS convex to plane, obtuse, slightly umbilicate, covered with small closely packed tufts of tawny fibers making the sur-

face appear almost granulate or muricate, the yellow background of the pileus shows in the cracks. LAMELLAE adnexed, yellow, becoming rusty. STEM concolorous, stuffed or hollow, densely clothed with scaly fibers which are more or less squarrose, with tufts of bright yellow mycelium at the base. ANNULUS slight of the character of that of the Squarrosae. SPORES 4–5x7–8μ.

The plant is reported from Michigan by Longyear.

IV. Small plants with an hygrophanous pileus and a membranous annulus.

The plants are closely related to the Pholiota togularis type but grow on logs and stumps.

18. Type of Pholiota marginata.

Pholiota marginata, Batsch. Pls. LIV and LV.

MARGINED PHOLIOTA.

The plants are common on decayed logs in damp woods late in the autumn. In our region they are usually almost even on the margin of the pileus and very rarely sufficiently striate to justify their name. In this respect they agree with the New York type which Peck has described as Pholiota marginella. The forms are very various as the illustrations show but we have not been able to separate any of the allied species such as Pholiota unicolor or Pholiota mutabilis. The plants in Pl. LIV, C closely resemble those in Hard's photograph of Pholiota unicolor but they do not agree with the plant figured in Flora Danica.

PILEUS watery brown or honey colored, from incurved to convex or expanded, smooth, margin even or slightly striate, sometimes recurved. LAMELLAE adnate or decurrent toothed, watery cinnamon becoming rusty ochraceous. STEM equal or slightly tapering upward, hollow, sometimes inflated, more or less white pruinose, fibrous striate, somewhat mealy at the apex and white velutine at the base. ANNULUS membranous, usually adhering to the stem but sometimes to the margin of

the pileus. SPORES elliptic or obovate 5—6x8—10μ. (Sylloge gives 3–4x6–7μ or 4–6x10–14μ.)

Note. A number of small species of Pholiota with hygrophanous pilei growing on decayed logs have been reported from this country.

Pholiota mutabilis, Schaeff. Somewhat larger than Pholiota marginata with the stem covered with squarrose scales. Morgan and Hard report it from Ohio.

Pholiota unicolor, Fl. Dan. Similar to Pholiota marginata but smaller and lamellae decurrent. Reported from Ohio by Morgan and Hard.

Pholiota marginella, Pk. Mus. Bull., 122, pp. 157–158. It is distinguished from Pholiota marginata by "its even fibrillose margin, adnexed lamellae and paler uniformly colored stem."

Pholiota autumnalis, Pk. N. Y. State Mus. Rep't 23, p. 92 (as Naucoria) and Mus. Bull. 122, p. 156. Glatfelter reports it from St. Louis with the remark "It appears to me the same as Pholiota marginata."

Pholiota discolor, Pk., N. Y. State Mus. Rep't 25, p. 78, is characterized by the change of color from cinnamon rufus when moist to bright ochraceous yellow when dry. Otherwise like Pholiota marginata.

19. Type of Pholiota confragosa.

Pholiota confragosa, Fr. Pl. XLI, D. E.

The plants grew on a log at Neebish, Mich., in September. The enlargement, Pl. XLI, E, shows the peculiar white floccose covering of the pileus. It is different from that of any other species of Pholiota. The plants were brick red but a little duller than in the illustration in Fries, Icon, 105. Otherwise the illustration represents our plants well. The plants photographed are young and smaller than the average.

PILEUS convex becoming plane, obtuse, ground color almost brick red, covered with a white flocculose coat easily rubbed off and which disappears when the plants become old, margin slightly striate when moist. LAMELLAE adnate, narrow, edge eroded. STEM equal, straight or slightly flexuous, ground color similar to the pileus, peronate with a fibrous scaly white coat which terminates in a spreading membranous white ring. SPORES rusty 5–6x7–8μ.

Note. Two species described by Peck belong to the Hygrophana but differ from the above types.

Pholiota acericola, Pk., N. Y. State Mus. Rep't 25, p. 77, is a large plant with a yellow or smoky yellow rugosely reticulated pileus, lamellae becoming longitudinally wrinkled when dry, a fibrillose whitish stem sometimes enlarged at the base and a large white deflexed annulus. It is reported from Michigan by Kauffman.

Pholiota cerasina, Pk., N. Y. State Mus. Rep't 26, p. 57 is a plant about the size of the former with a marked amygdaline odor.

C. Very small species growing among mosses.

We have no photographs of species in this division. They are known by the rusty spores and the annulus on the stem. Pholiota mycenoides, Fr. is reported from Michigan by Longyear. Pholiota pumila is in Farlow's Index and Peck has described Pholiota minima from New York state and reported Pholiota rufidula, Kalch, from Massachusetts.

INDEX OF THE SPECIES.

The species followed by a plate number are described and illustrated. The others are referred to in the notes.

EXPLANATION OF PLATES

Plate XXIV. Pholiota caperata, (Pers.) A. Mature plant of average size. B. Young plant with ovate cap covered with white flocci.

Plate XXV. Pholiota ..... A. Mature plant a little above average size showing the scaly bulbous stem, the ridges on the annulus and the erose denticulate gills. B. Part of the surface of a pileus showing the innate scaly fibers. C. Part of the annulus of a dried specimen x 4.

Plate XXVI. Pholiota howeana, Pk. Mature plant.

Plate XXVII. Pholiota praecox, (Pers.) A. Mature plant showing the gill surface. B. Upper side of a pileus. C. Plant with the annulus tearing from the stem. D. Plant with an umbonate pileus and twisted striate stem.

Plate XXVIII. A. B. Pholiota, praecox (Pers.) C—F. Pholiota vermiflua, Pk. A. Plant with an annulus on the stem. B. Underside of a pileus showing veil separating from the margin. C. Mature plant. D. Section showing the thick flesh and ventricose gills. E. Gill surface. F. Surface of a pileus.

Plate XXIX. Pholiota dura, (Bolt.) A. Mature plant with expanded pileus. B. Younger plant showing membranous annulus. C. Plant showing the gill surface.

Plate XXX. Pholiota erebia, Fr. A. Cluster of plants showing adhering scaly stems and membranous annulus. B. Underside of a pileus showing the gill surface and hollow stem. C. Upper side of a pileus showing rugose surface. D. Section showing flesh and gills.

Plate XXXI. Pholiota ombrophila, Fr. Two mature plants.

Plate XXXII. Pholiota togularis (Bull.) A. Old plants with depressed pilei. B. Younger plants some of which grew on sticks.

Plate XXXIII. A. Pholiota temnophylla, Pk. The under side of the pileus shows the shape of the gills. B. Pholiota terrigena, Fr. Cluster of young plants.

Plate XXXIV. Pholiota angustipes, Pk. A. Cluster showing hemipheric pilei and scaly stems tapering downward. B. Parts of plants showing the gill surface and the squamose-dotted pileus.

Plate XXXV. A. Pholiota squarrosa, Muell. Two plants showing the stems and the under surface of the pilei. B. Pholiota Squarrosa, var. verruculosa, Lasch. Two plants showing the sharp verrucose scales on the pilei and shaggy scales on the stems.

Plates XXXVI and XXXVII. Pholiota squarrosoides, Pk. A cluster of young plants is shown on plate XXXVI and a cluster of older plants giving different view on plate XXXVII.

Plates XXXVIII and XXXIX. Pholiota aurivella, Batsch. The first shows the surface of the pileus and the stem of a medium sized plant, the second shows the under side of two plants with the gill surface and the stems smooth above and scaly below.

Plate XL. Pholiota adiposa, Fr. The under side of three plants taken from a large cluster showing the gill surface and the scaly stems which are darker colored below.

Plate XLI. A. B. Pholiota tuberculosa, Fr. Plant showing the bulbous scaly stem, and the surface of a pileus. Both taken from dried plants. C. A plant of Pholiota flammans, Fr. D. E. Pholiota confragosa, Fr. Three plants natural size, the underside of a pileus and one of the plants x 4 showing the floccose surface.

Plates XLII and XLIII. Pholiota albo-crenulata, Pk. XLII. A. The underside of a mature plant. B. Part of the gill surface x 4 showing the white granules on the edges of the lamellae. XLIII. A. The gill surface of a mature plant. B. The surface of a pilus. C. Young plant showing the method of tearing of the veil.

Plate XLIV. Pholiota spectabilis, Fr. A. Young plant showing the tearing of the veil. B. Young plant showing the fibrous surface of the pileus. C. Section showing the thick flesh and narrow gills. D. Very young plants covered with the universal veil.

Plate XLV. Pholiota comosa, Fr. A. Surface of the pileus covered with white hairy scales. B. Section showing flesh and gills. C. Mature plant with an elongated stem. D. Part of a plant reduced one half showing the bulbous stem.

Plates XLVI and XLVII. Pholiota heteroclita, Fr. The first plate shows a mature plant and the second a section of a plant with an eccentric stem, thick white flesh and broad lamellae, also part of the surface of a pileus showing the scales.

Plate XLVIII. Pholiota luteofolia, Pk. A. Two old plants with depressed pilei and a section of another plant showing wide gills. B. Underside of a pileus showing the gills and the stem hollow from age. C. Part of a pileus showing the scaly surface.

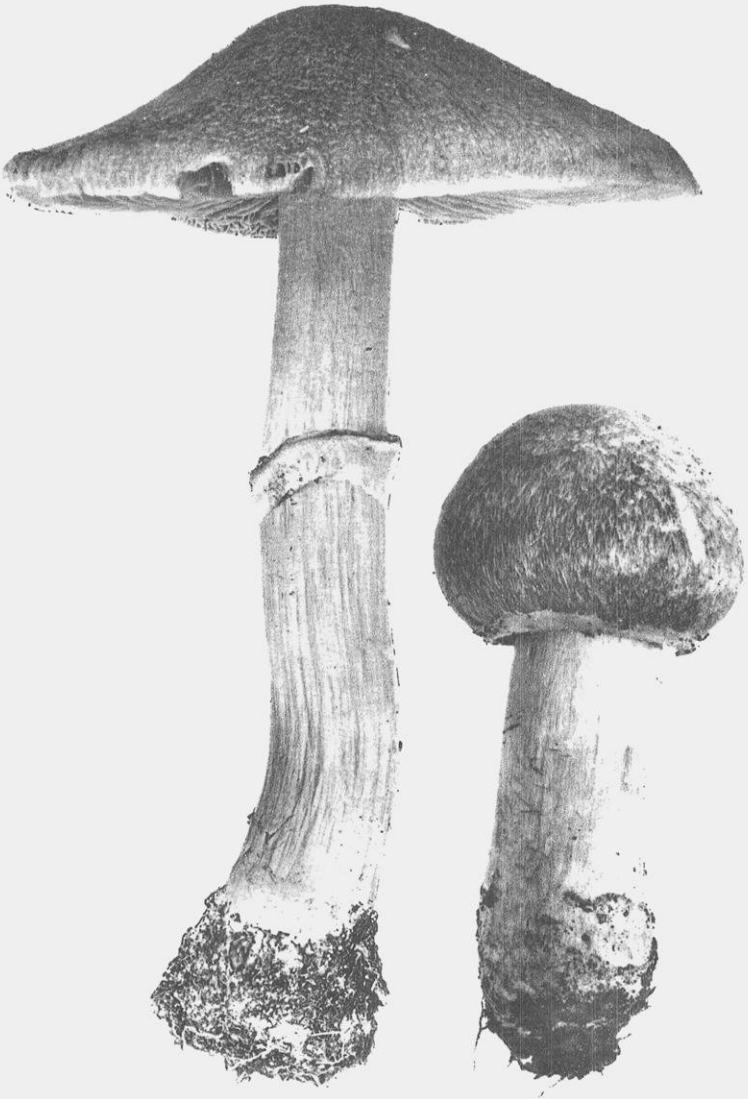
Plate XLIX. Pholiota aegerita, Brigant. Two mature plants.

Plate L. Pholiota lutea, Pk. A. Cluster of large plants. B. Young plants covered with the universal veil.

Plate LI. Pholiota erinaceëlla, Pk. Plants of various ages showing the shape at the different stages of development and the bristly scaly universal veil.

Plates LII and LIII. Pholiota muricata, Fr. In plate LII, A shows a cluster of plants growing from a piece of rotten wood, the upper and under surface of the pileus and a young plant with the veil separating from the pileus, B shows plants with long straggling stems. Plate XXX shows plants in different stages of development with bunches of mycelium at the base of the stems.

Plates LIV and LV. Pholiota marginata, Batsch. Plate LIV. A. A very large plant. B. Young pileus showing separation of the veil. C. Cluster of plants with inflated white pruinose stems. D. Three plants growing on bark. Plate LV. Plants showing different stages of development.



A B

PHOLIOTA CAPERATA (PERS.)

C

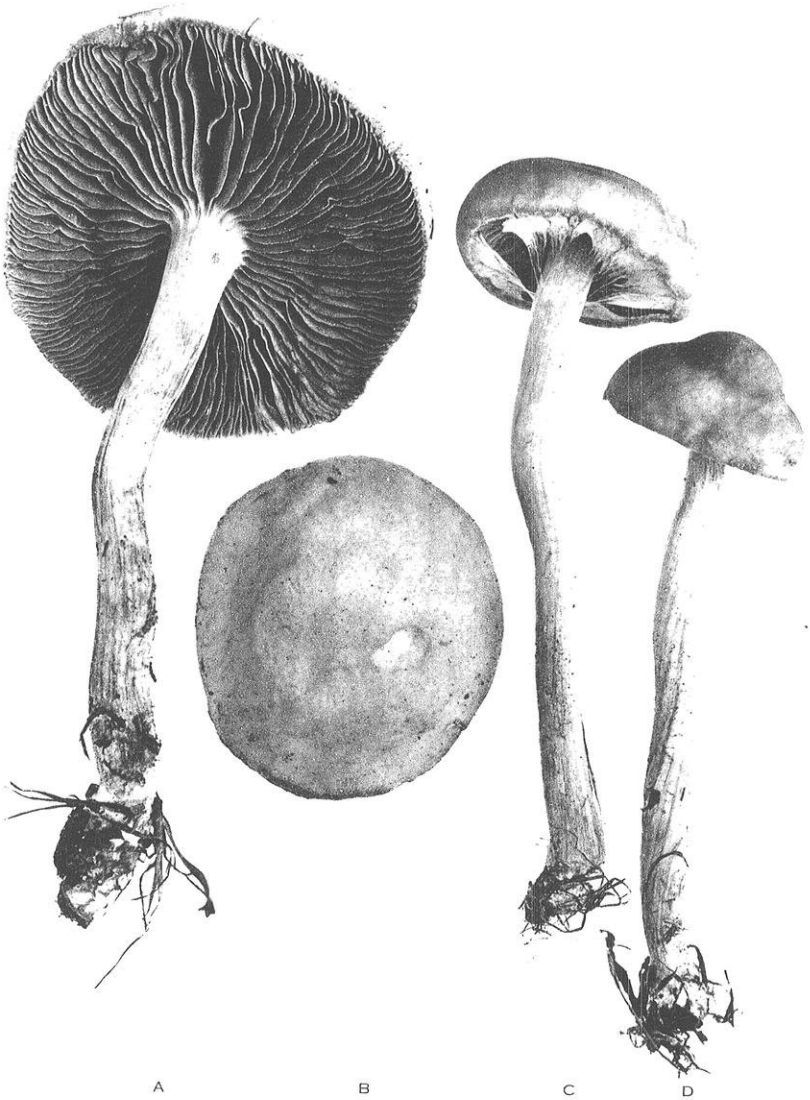
B

A

PHOLIOTA ?

PHOLIOTA HOWEANA, PK. (?)

COCKAYNE, BOSTON

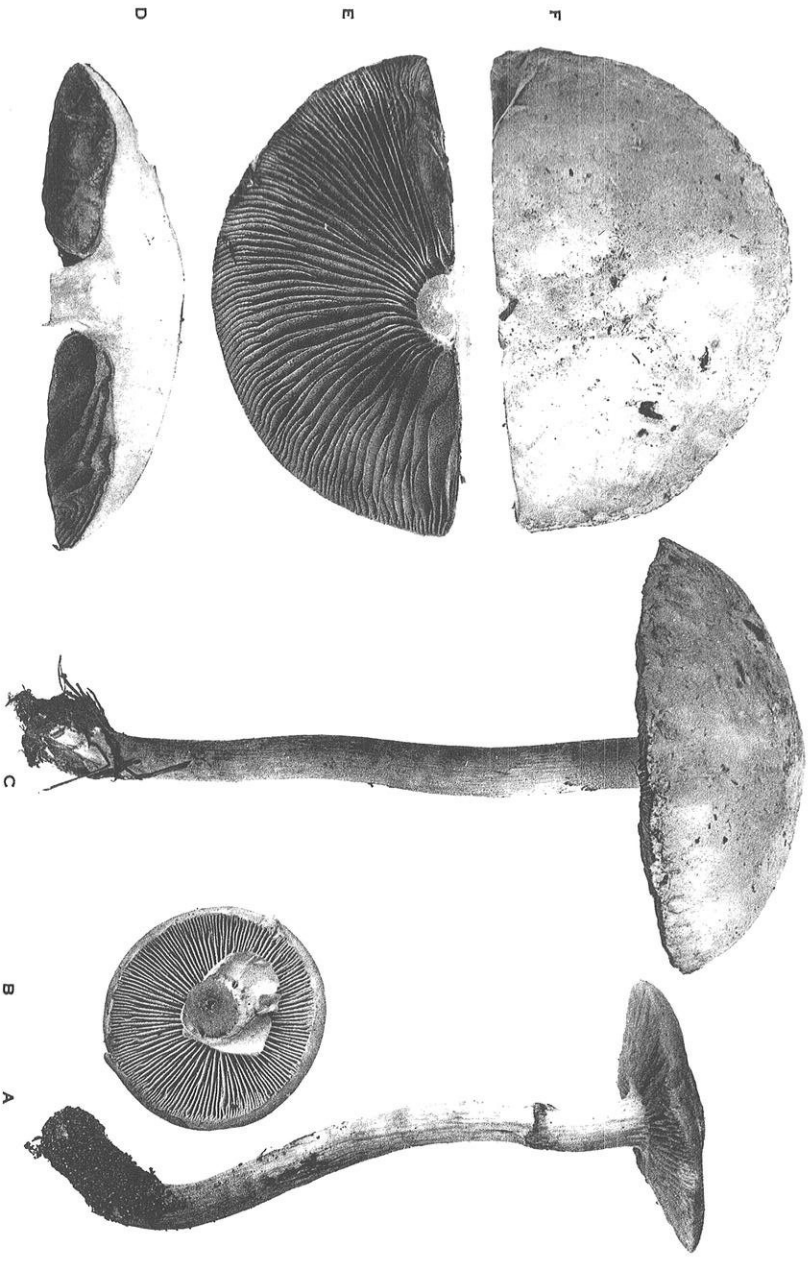


A B C D

PHOLIOTA PRAECOX. (PERS.)

HARPER—PHOLIOTA

COCKAYNE, BOSTON



A. B. PHOLIOTA PRAECOX (PERS.
C. D. E. F. PHOLIOTA VERMIFLUA, PK.

HARPER—PHOLIOTA

COCKAYNE, BOSTON

PHOLIOTA DURA. BOLT.

HARPER—PHOLIOTA

COCKAYNE, BOSTON

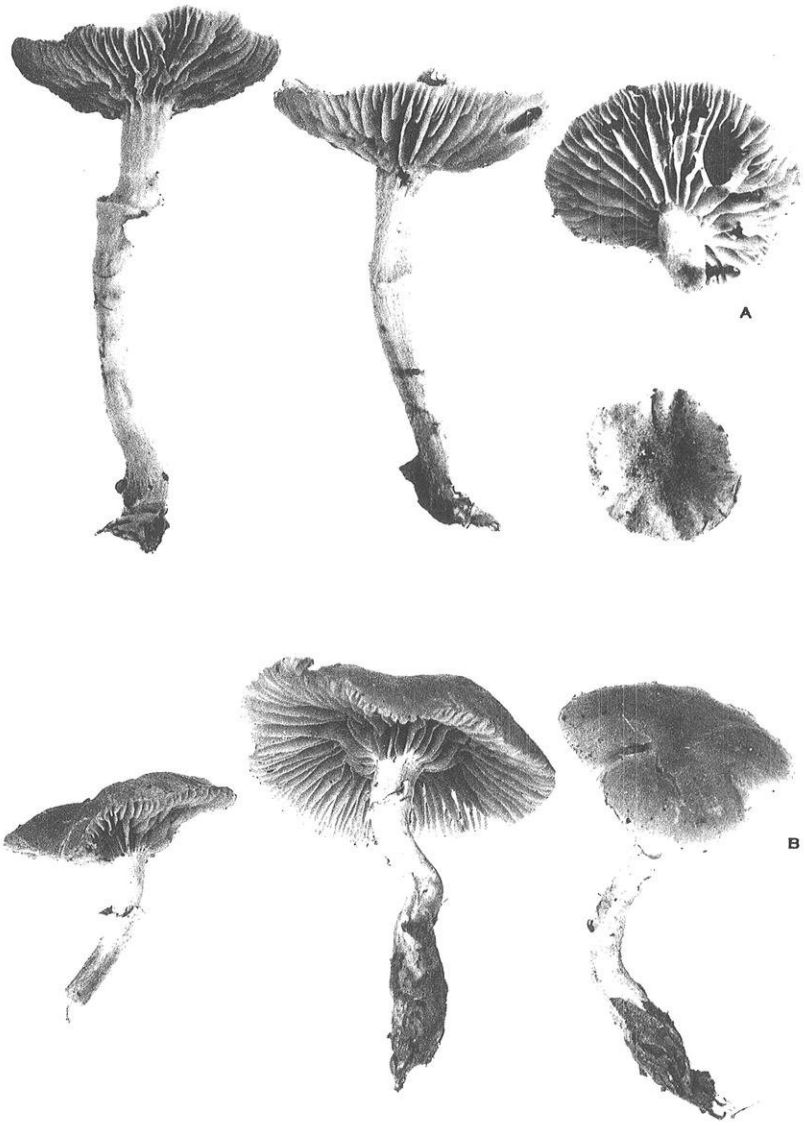
PHOLIOTA EREBIA. FR.

HARPER—PHOLIOTA

COCKAYNE, BOSTON

PHOLIOTA OMBROPHILA, FR

COCKAYNE, BOSTON



PHOLIOTA TOGULARIS (BULL.)

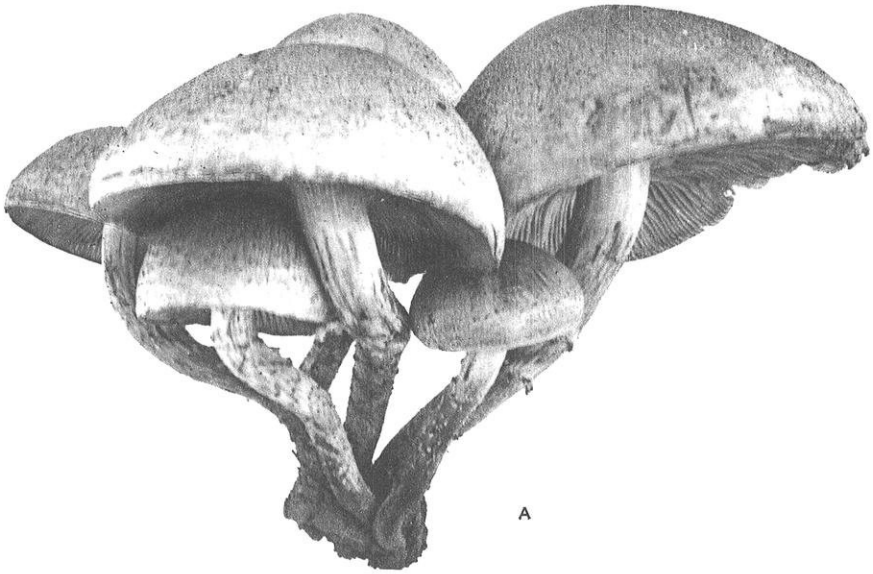
COCKAYNE, BOSTON

A

B

A PHOLIOTA TEMNOPHYLLA, PK.
B PHOLIOTA TERRIGENA, FR.

COCKAYNE, BOSTON

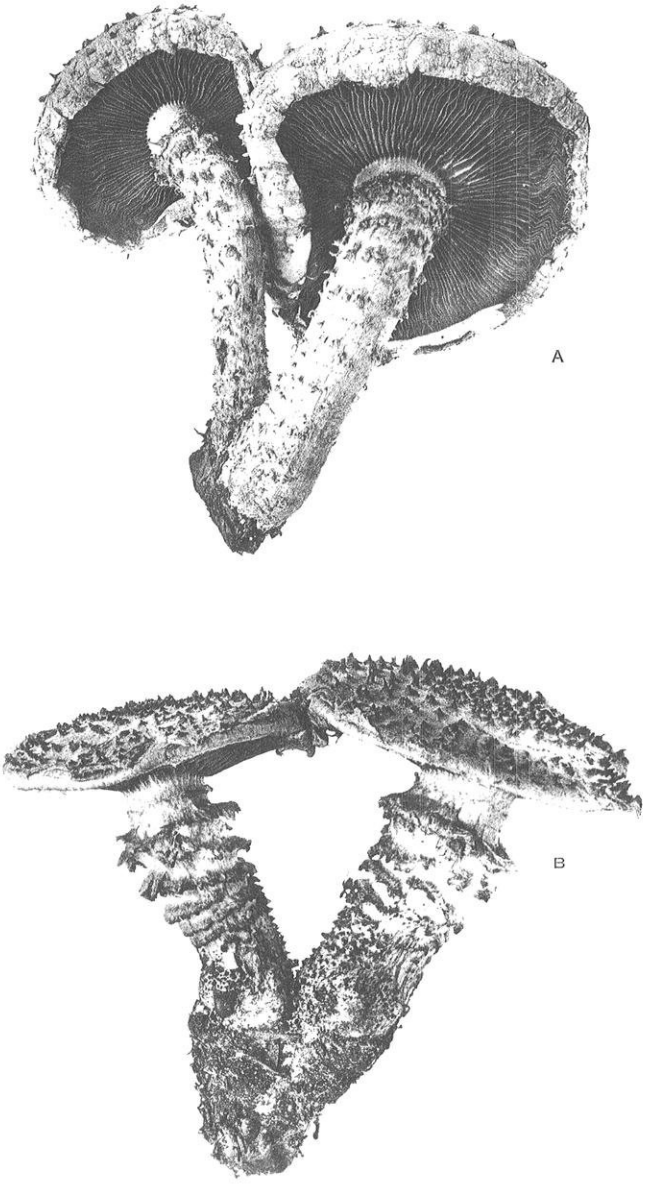


A

B

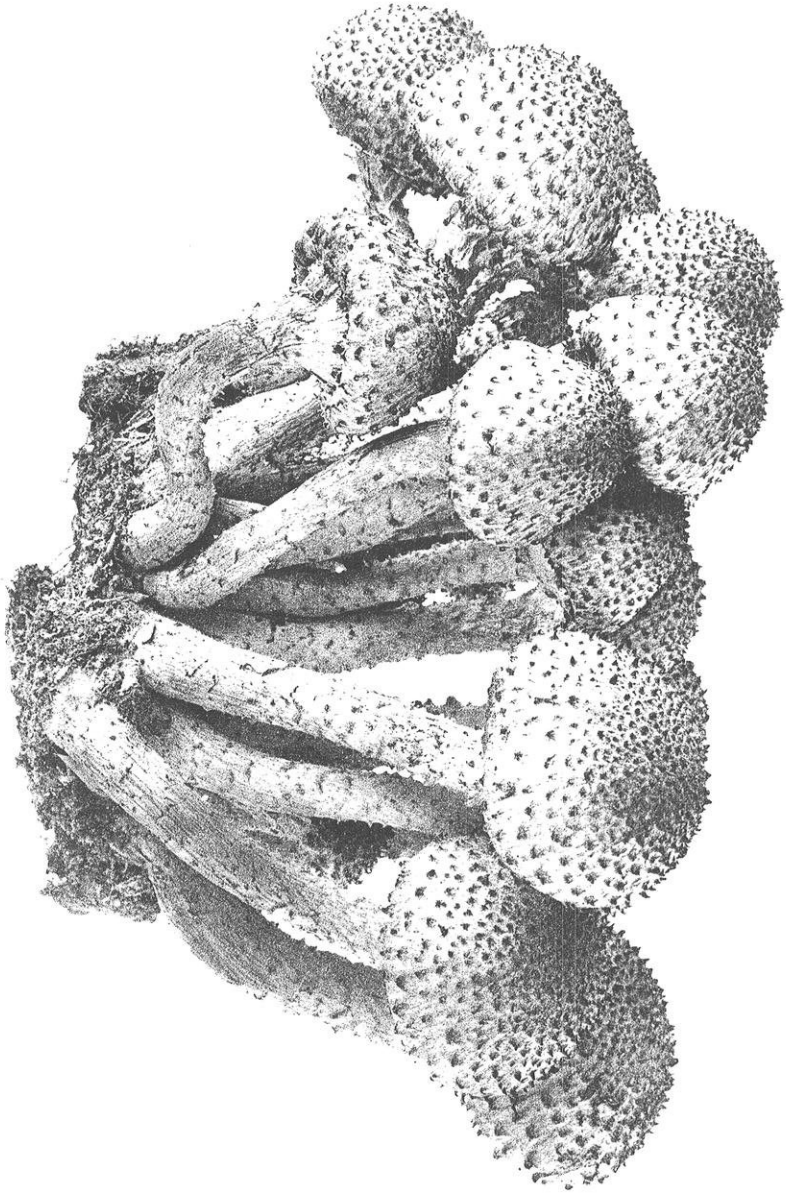
PHOLIOTA ANGUSTIPES, PK.

COCKAYNE, BOSTON



A PHOLIOTA SQUARROSA, MUELL.
B PHOLIOTA SQUARROSA, VAR. VERRUCULOSA, LASCH.

COCKAYNE, BOSTON

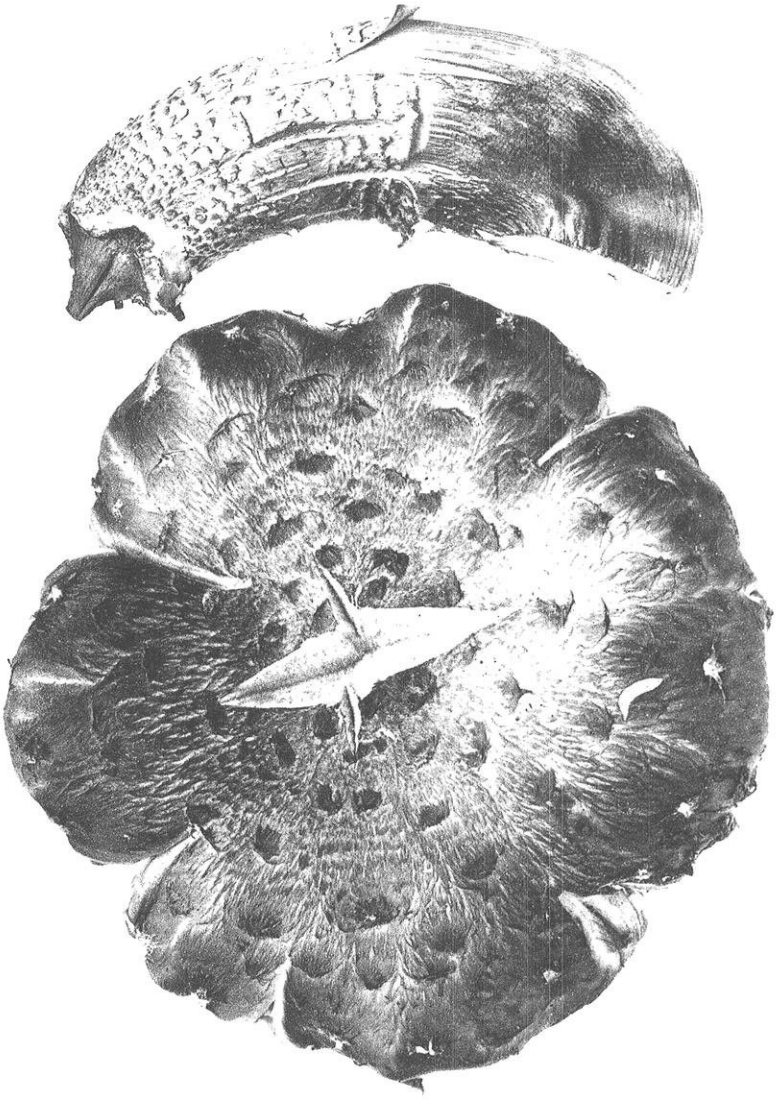


PHOLIOTA SQUARROSOIDES. PK.

COCKAYNE, BOSTON

PHOLIOTA SQUARROSOIDES, PK.

COCKAYNE, BOSTON



PHOLIOTA AURIVELLA, BATSCH

HARPER—PHOLIOTA

COCKAYNE, BOSTON

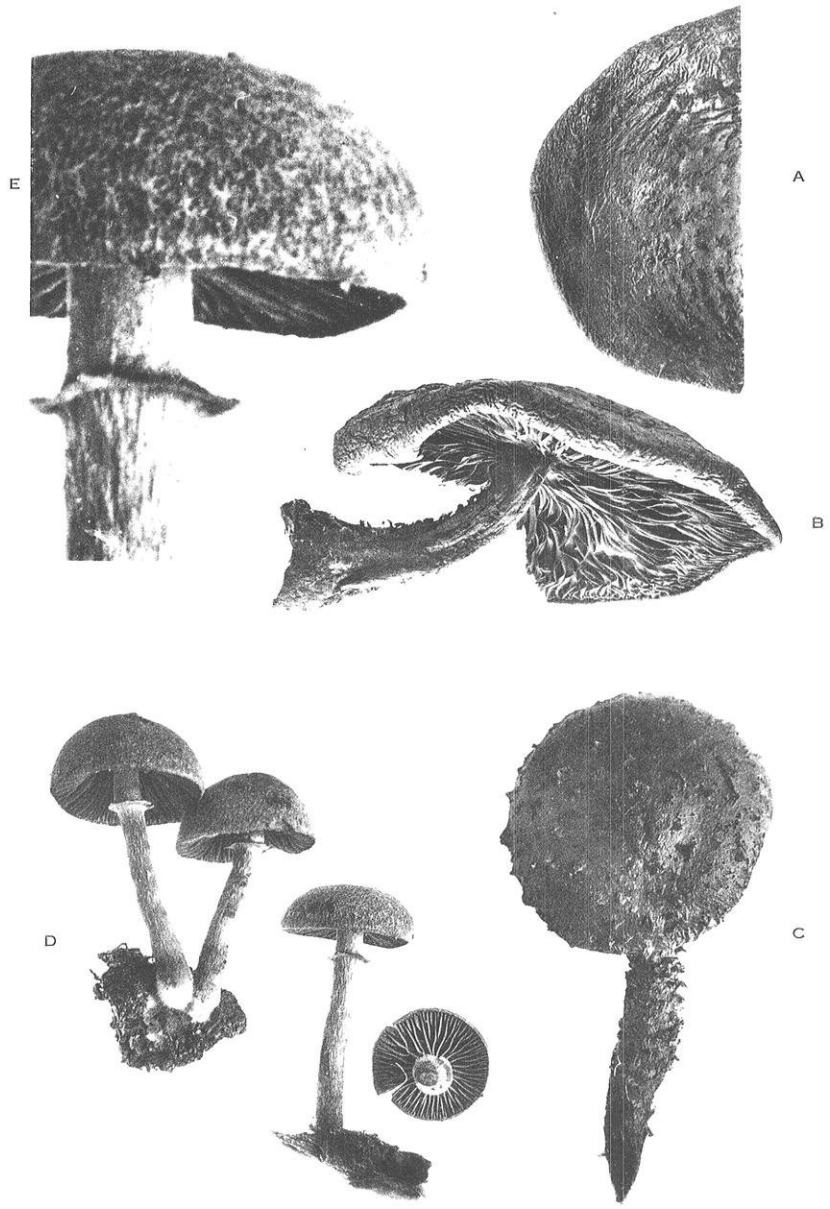
PHOLIOTA AURIVELLA, BATSCH

COCKAYNE, BOSTON

PHOLIOTA ADIPOSA. FR

HARPER—PHOLIOTA

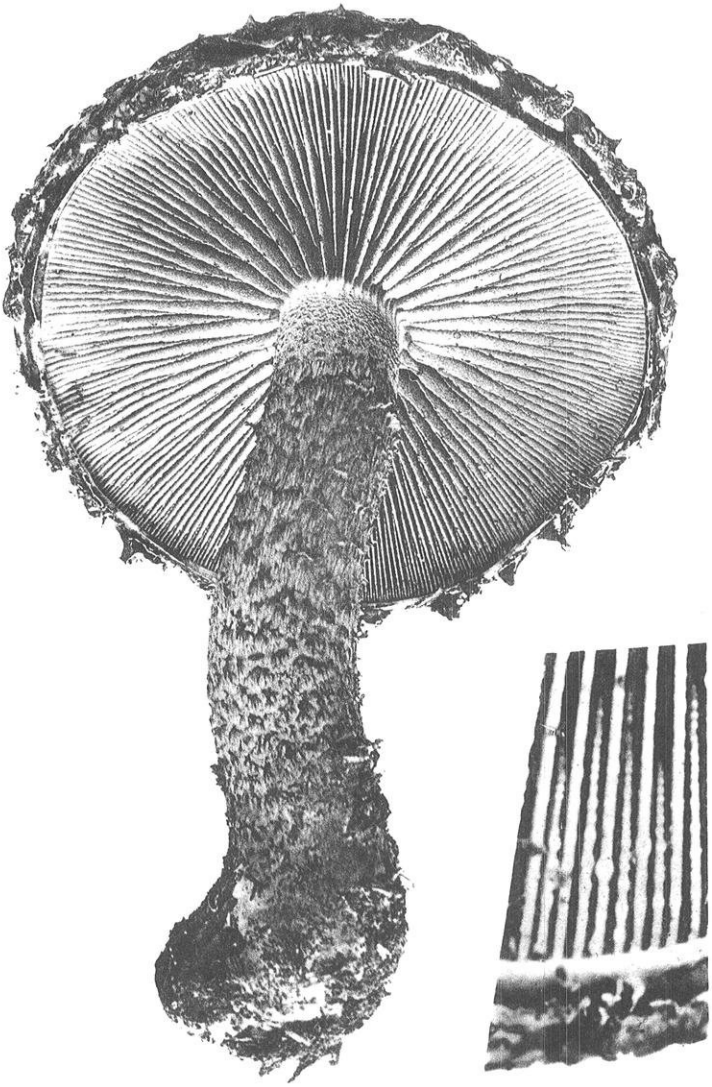
COCKAYNE, BOSTON



A B PHOLIOTA TUBERCULOSA, FR.
C PHOLIOTA FLAMMANS, FR.
D E PHOLIOTA CONFRAGOSA, FR.

HARPER—PHOLIOTA

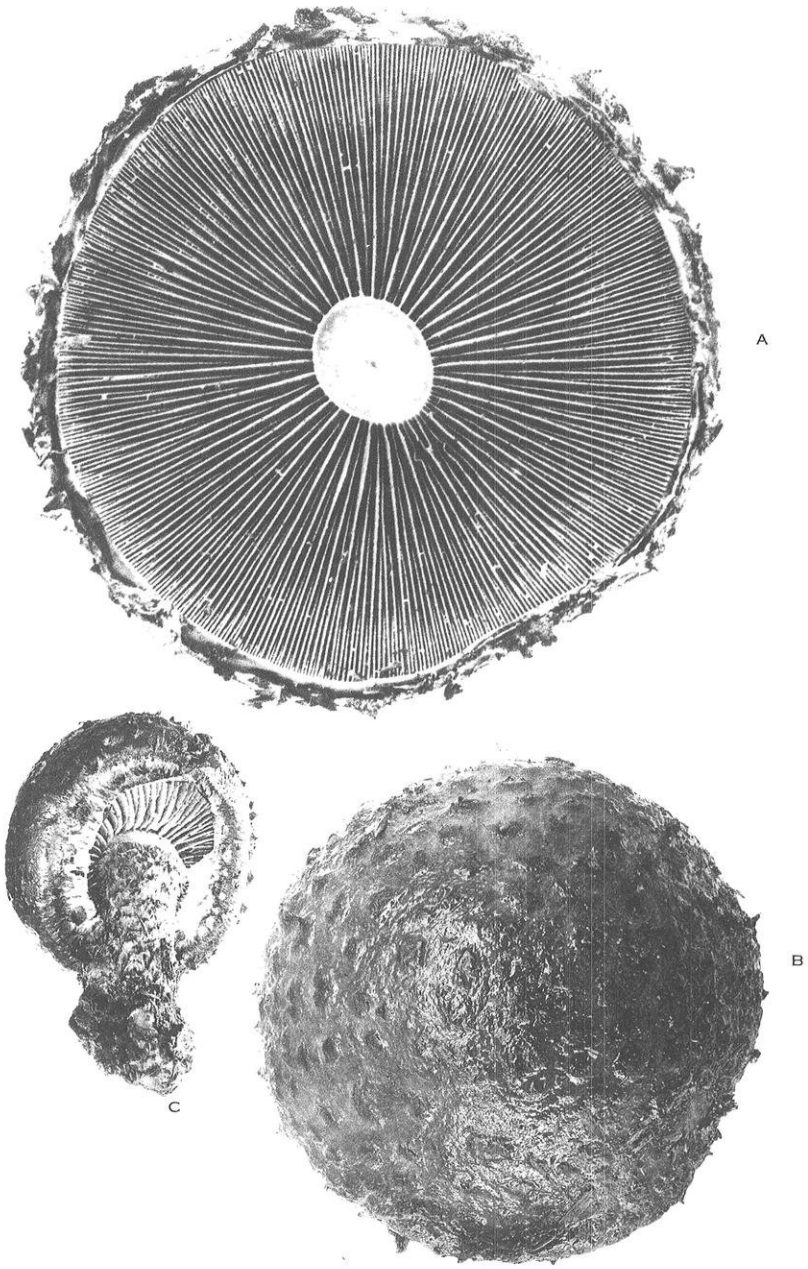
COCKAYNE, BOSTON



A B

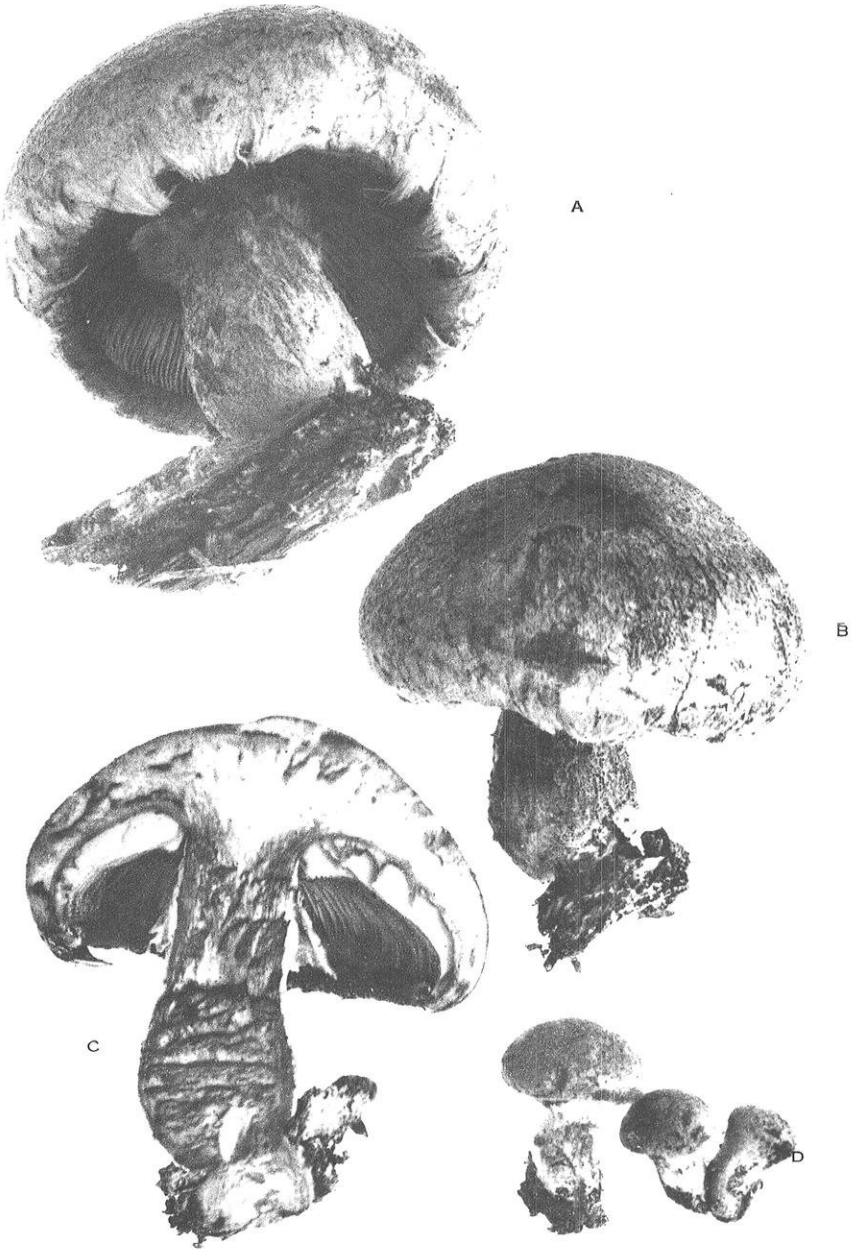
PHOLIOTA ALBO-CRENULATA, PK.

COCKAYNE, BOSTON



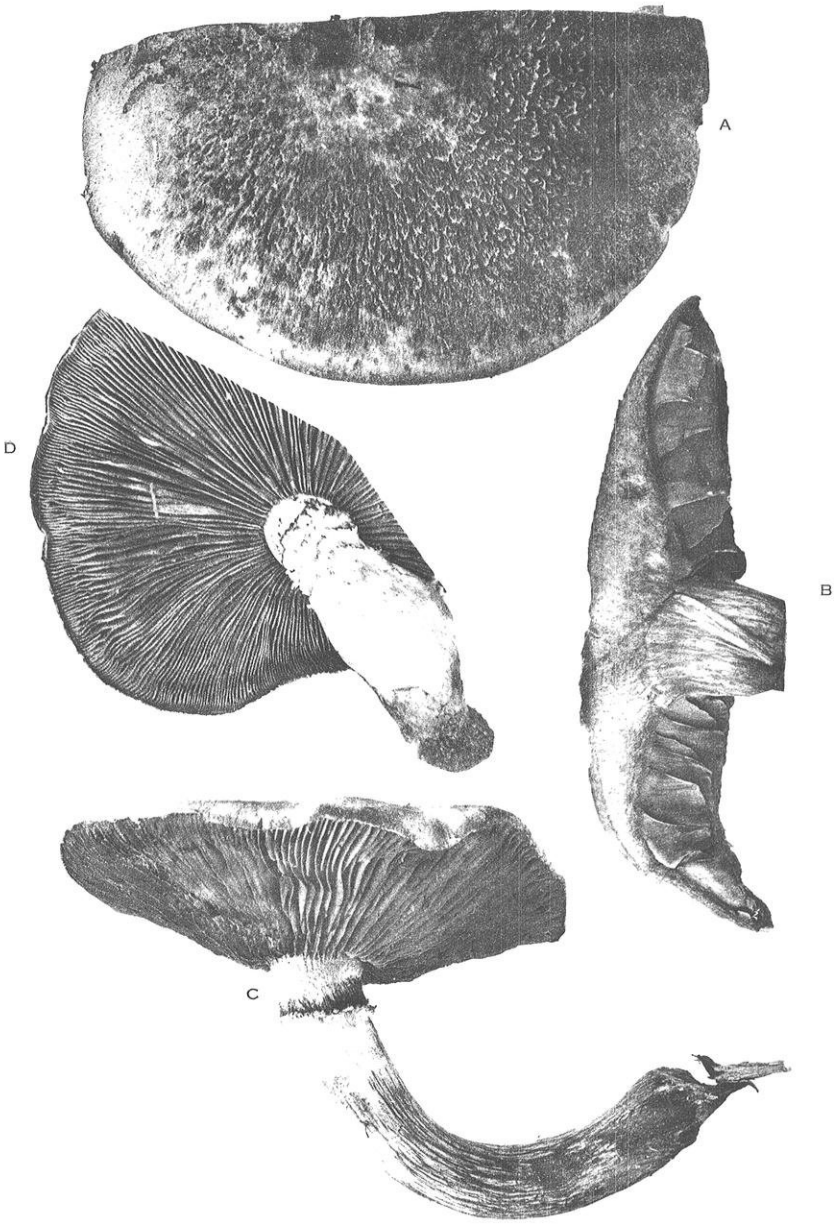
PHOLIOTA ALBO-CRENULATA, PK

COCKAYNE, BOSTON



PHOLIOTA SPECTABILIS, FR.

COCKAYNE, BOSTON



PHOLIOTA COMOSA, FR.

COCKAYNE, BOSTON

PHOLIOTA HETEROCLITA. FR.

COCKAYNE, BOSTON

B

A

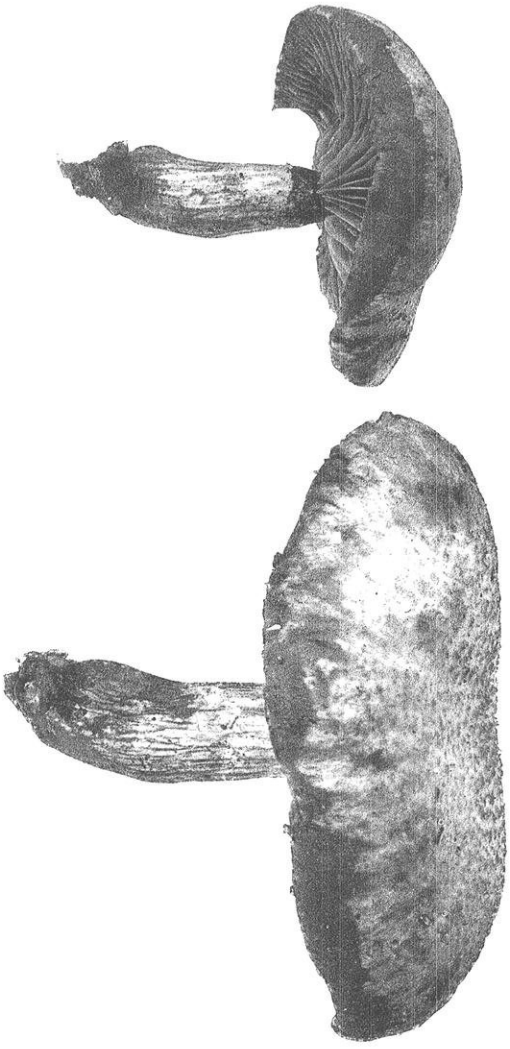
PHOLIOTA HETEROCLITA, FR.

HARPER—PHOLIOTA

COCKAYNE, BOSTON

PHOLIOTA LUTEOFOLIA, PK.

COCKAYNE, BOSTON

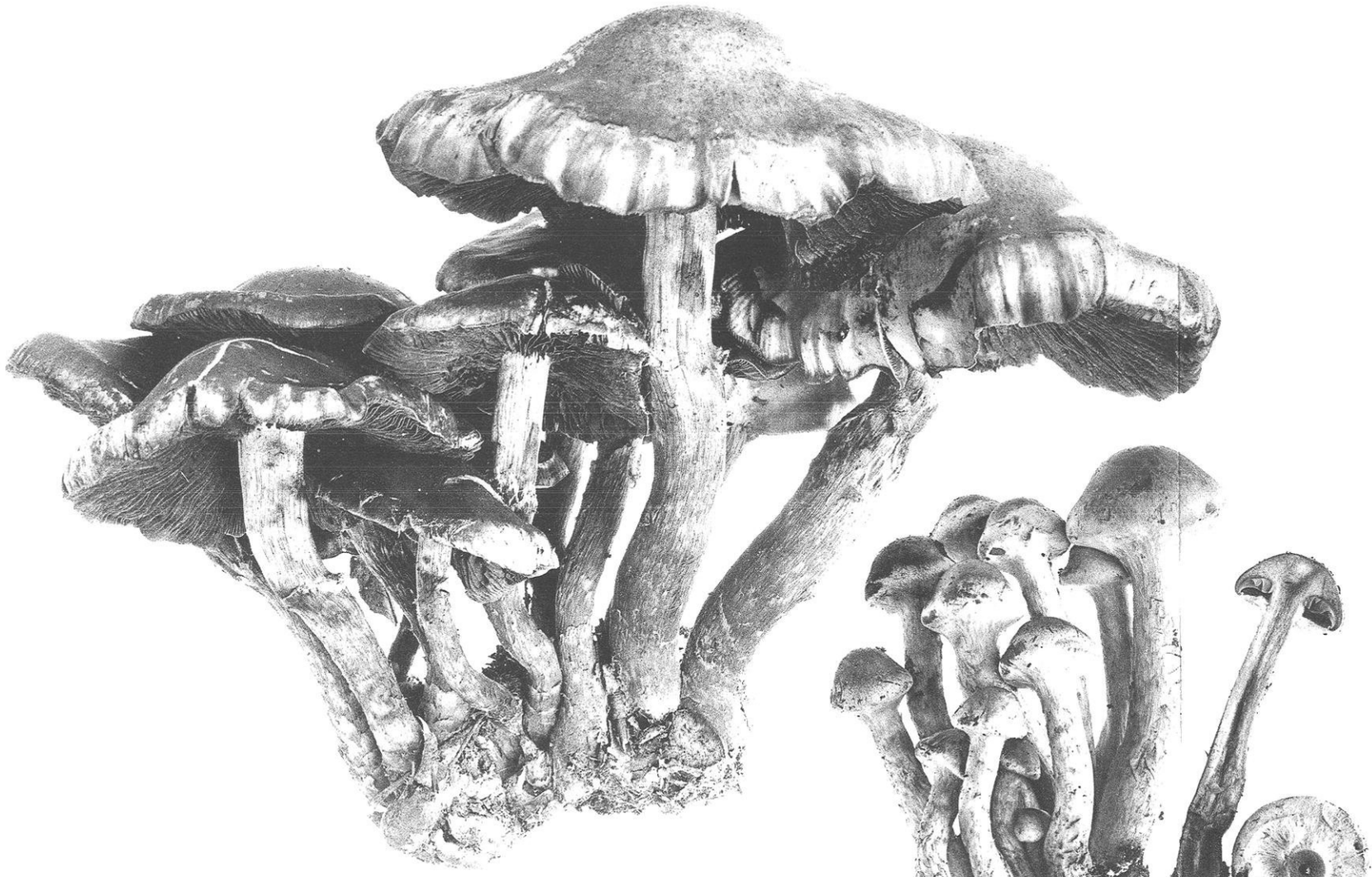


B

A

PHOLIOTA AEGERITA, BRIGANT.

COCKAYNE, BOSTON

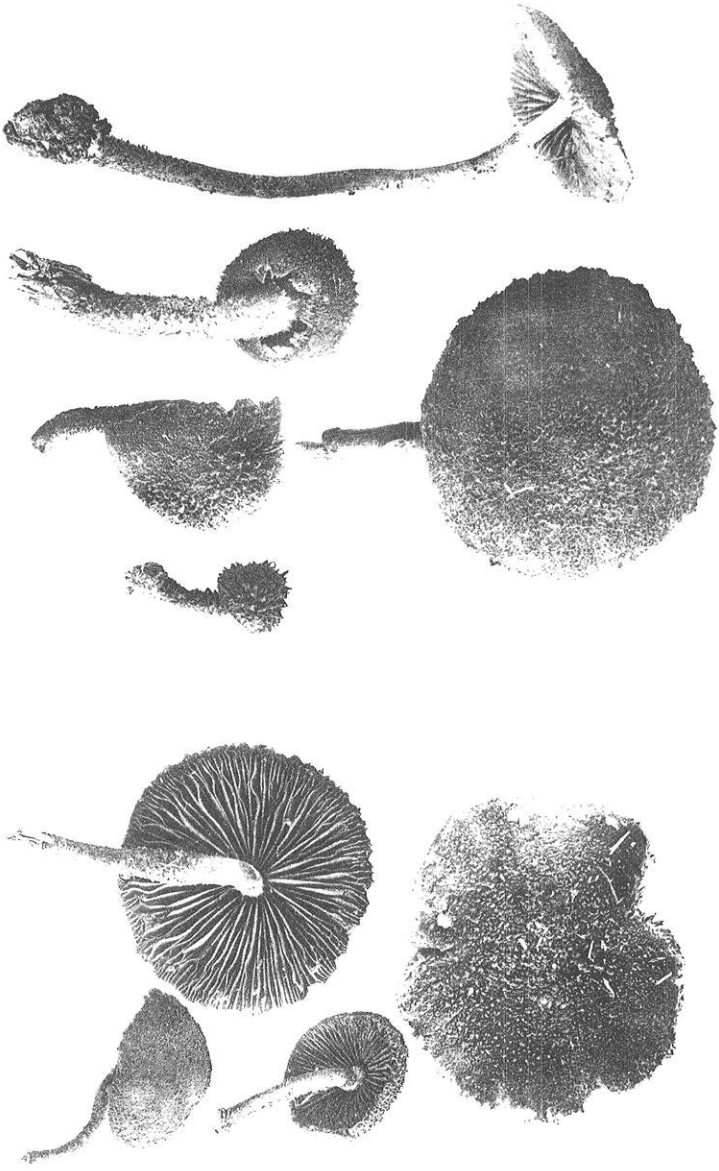


A

B

PHOLIOTA LUTEA, PK.

COCKAYNE, BOSTON



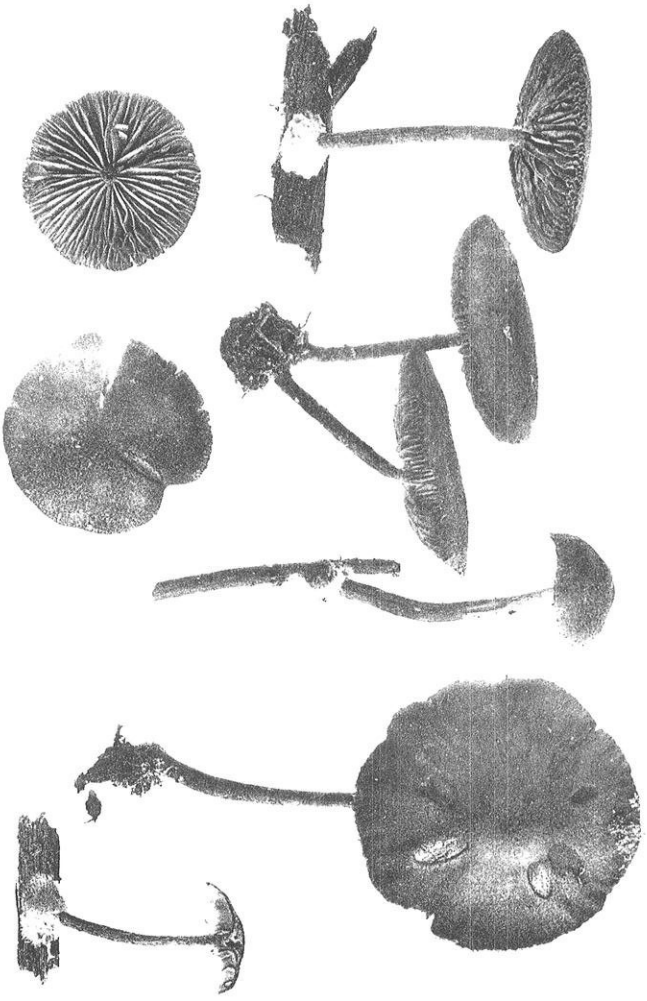
PHOLIOTA ERINACEELLA. PK.

HARPER—PHOLIOTA

COCKAYNE, BOSTON

PHOLIOTA MURICATA, FR.

COCKAYNE, BOSTON



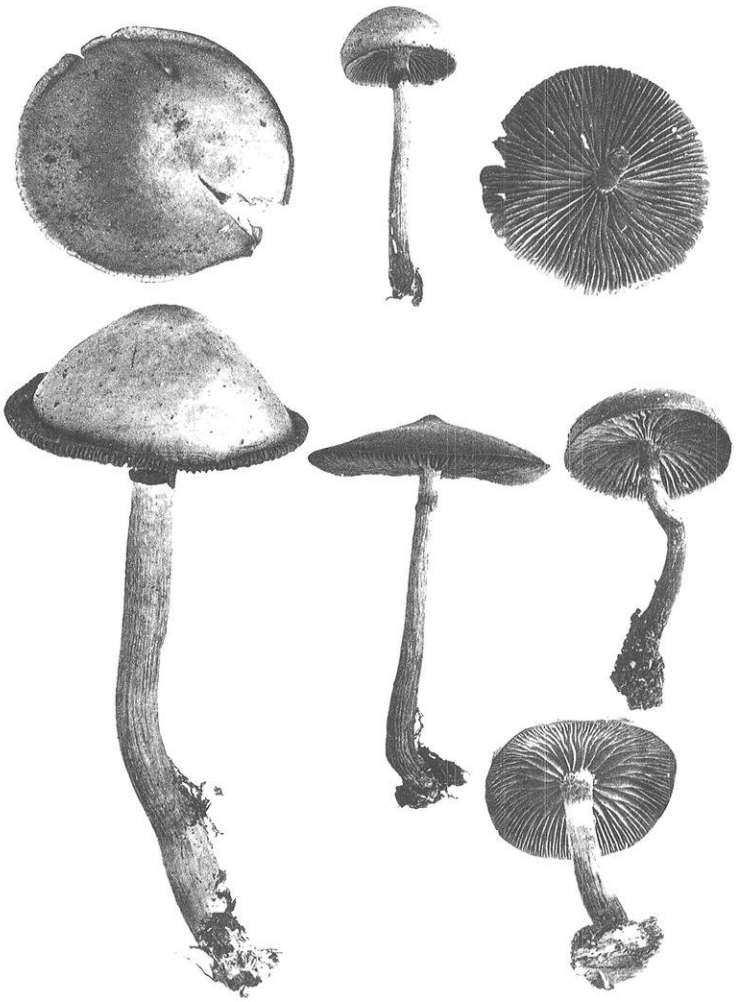
PHOLIOTA MURICATA. FR.

COCKAYNE, BOSTON

PHOLIOTA MARGINATA. BATSCH.

HARPER—PHOLIOTA

COCKAYNE, BOSTON



PHOLIOTA MARGINATA, BATSCH.

COCKAYNE, BOSTON

THE WALDEN INVERSION—A CRITICAL REVIEW.

A. F. McLeod.

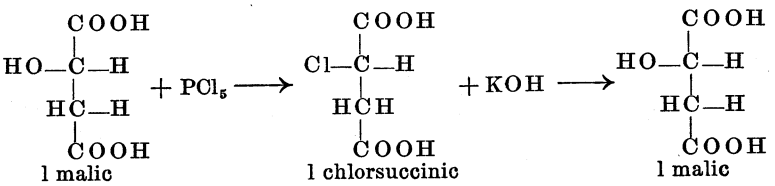
In 1891, Walden[1] asserted that by means of a simple cycle, he could pass directly from one optically active acid to its antipode. By treatment of natural l malic acid, for example, with phosphorus pentachloride, he obtained a laevorotatory chlorsuccinic acid, in which, by subsequent treatment with moist silver oxide, he was able to substitute hydroxyl (OH) for chlorine (Cl), and recover the hydroxy acid used as starting material. To his great surprise, however, the malic acid thus obtained was strongly dextrorotatory. When Walden used, in place of moist silver oxide, stronger bases, e. g. potassium or sodium hydroxide, he recovered the original l malic acid. An inversion in optical activity must have occurred, therefore, in his first cycle either with phorphorus pentachloride or with silver oxide.

This work was ridiculed by all of the leading chemists at that time, since it was entirely out of harmony with the ideas advanced by Vant' Hoff and Le Bel. These men had pointed out that all of the optically active bodies studied by Pasteur had at least one asymmetric carbon atom (meaning by 'asymmetric,' that all of the atoms or groups attached to such a C atom were different each from the other). This idea had served to put the chemistry of the optically active bodies on a substantial and rational basis. Earlier chemists[2] had been forced to assign different structural formulae to optical insomers—for a long time the formula $CH_2OH–CH_2–COOH$ was given to 'fleishmilch-

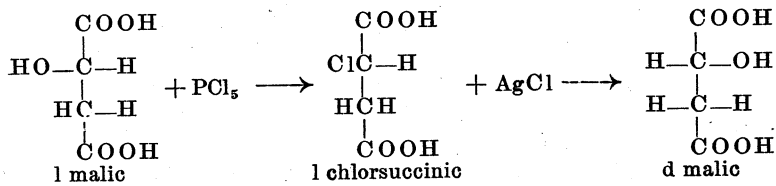
[1] Ber, 28, 1293.
[2] Wislicenus: Ann. 166, 6; Moldenhauer: Ann. 131, 323.

säure' (d lactic acid) to distinguish it from 'gährungs milchsäure' (dl lactic). According to Vant 'Hoff's idea, the difference in physical properties of optical antipodes could be indicated structurally in a simple manner by means of a different arrangement of the various groups around the asymmetric carbon atom.

To maintain a rational system it was necessary, of course, that in the simple exchange of one radical for another in an optically active compound by means of various reagents, no rearrangement of the atoms in space should occur—in other words, the substituted product should have a structure corresponding to that of the original material, as is represented by the following scheme:



Walden determined that these changes took place as indicated, both of which according to his latest work he regarded as normal.[3] He discovered, however, that by using moist silver oxide to replace the halogen, he always obtained an abnormal result (a product which demanded a rearrangement of the atoms in space). Such an abnormal result always lights the way to a new discovery, and demands an extension or revision of our present theory. Walden was, therefore, not slow in announcing that he had obtained an inversion in the optical activity of malic acid by means of a simple cycle in which OH was replaced by Cl using phosphorus pentachloride, and the halogen atom, in turn, was replaced by hydroxyl using moist silver oxide—whence the name, the 'Walden inversion.'



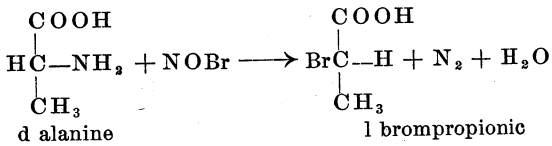
That such an inversion actually takes place is wonderful indeed and, in my opinion, still remains to be proved, in spite of the apparently absolute demonstration by Walden, Purdie, Fisher, McKenzie and many others. Some recent experimental work which I have done, indicates that the acid product obtained by the action of silver oxide in water solution on dl brompropionic acid (a change entirely analagous to the one given above) *has properties entirely different from those of ordinary dl lactic acid.* This evidence will be discussed at greater length in the experimental part of this paper. Since some confirmatory evidence in the absolute proof to the contrary is still lacking, let us assume here for simplicity, that the Walden inversion is an established fact.

For a long time Walden was misled by the fact that his chlorsuccinic acids gave an actual rotation opposite to that indicated by their sign i. e. d chlorsuccinic rotated laevo and vice versa. But he decided finally[4] that the action of phosphorus pentachloride on malic acid was normal, a conclusion which he deduced largely from theoretical and physico-chemical considerations. Having determined this, it followed as a matter of course that the action of potassium hydroxide was normal, while that of moist silver oxide was abnormal. The question as to whether the action of nitrous acid on asparaginic acid to give malic was normal or abnormal, Walden left open, since he was unable to determine. Having contributed a splendid and practically complete demonstration of the chemistry of the optically active malic acid series, Walden published the last of his five papers in 1899, and left this field of work.

With the exception of a small but very significant contribution by Purdie and Williamson,[5] nothing of vital importance was done on the Walden inversion from 1899 until March, 1907, when Emil Fisher published his first article, "Zur Kenntnis der Waldensche Umkehrung."[6] In synthesizing various optically active polypeptides, Fisher was forced to determine absolutely

[3] Berichte 32, 1855.
[4] Ber, 32, 1833 and 1855.
[5] Trans. L. Ch. Soc. [1896] p. 838.
[6] Ber, 40, 1051.

by experiment whether there was anything in the idea of the Walden inversion i. e. whether by use of certain reagents, he might obtain, not the corresponding substituted product, but its optical antipode. This could only be determined by careful experiment, since the sign of actual rotation whether dextro or laevo, is no positive indication as to whether the body is really a (d) or an (l) derivative. Fisher not only confirmed the work of Walden by showing that an analagous inversion takes place with α brompropionic acid by the successive action of phosphorus pentachloride and moist silver carbonate, but he also discovered another case where the change was accompanied by inversion, namely the conversion of optically active alanin into brompropionic acid, using nitrosyl bromide (NOBr) as the brominating agent.



Fisher rigidly established that the Walden inversion was confined to these two phases: Phase 1—replacement of Cl by OH by means of silver oxide or other bases of the same type. Phase 2—replacement of amino (NH_2) group by halogen, using nitrosyl halide as agent. That the action of phosphorus pentachloride was normal, while that of nitrosyl bromide was abnormal, he established as follows:

d iactic acid + PBr_5 ⟶ d brompropionic acid (dextrorotary)

d lactic ethyl ester + PBr_5 ⟶ d brompropionic ethyl ester (dextrorotatory)

d alanin + NOBr ⟶ l brompropionic acid (laevorotatory)

d alanin ethyl ester + NOBr ⟶ d brompropionic ethyl ester (dextrorotatory).

Other evidence of course was furnished, but the main point in Fisher's proof was the comparison of results obtained by various reagents on the free acids and their corresponding esters. If these two actions were analagous, the substituting agent reacted normally; if different products were obtained in the two actions, an inversion must have occurred in the case of

the free acid. That the inversion took place with the free acid rather than with the ester, Fisher decided from the fact that esters were less inclined to optical reversals than the corresponding acids. Having established as above that the action of phosphorus pentachloride or pentabromide resp. was normal, it followed that the subsequent action of potassium hydroxide on the brom acid, thus formed from lactic, was normal, while that of moist silver carbonate on the same brom acid was abnormal just as Walden finally decided. The correctness of the above reasoning Fisher also established as follows by direct experiment.

d brompropionic acid + KOH $\longrightarrow$ d lactic acid (— Zn salt)

d brompropionic acid + Ag_2O $\longrightarrow$ l lactic acid (+ Zn salt)

d brompropionyl glycin + Ag_2O $\longrightarrow$ d lactic ester (Free acid obt. by hydrolysis gave negative zinc s⩾t).

The actions of ammonia and of nitrous acid (nitrous fumes) were held to be normal from a consideration of the following reactions:

d brompropionic acid + NH_3 $\longrightarrow$ d alanin (dextrorotatory)

d brompropionic ethyl ester + NH_3 $\longrightarrow$ d alanin ethyl ester (dextrorotatory)

d alanin + N_2O_3 $\longrightarrow$ d lactic acid (Negative zinc salt)

d alanin ethyl ester + N_2O_3 $\longrightarrow$ d lactic ethyl ester (Free acid obt. by hydrolysis gave negative zinc salt).

Walden had tried the action of ammonia on chlorsuccinic acid but failed to get asparaginic acid. Fisher, by repeating Walden's experiment later,[7] was able to isolate a small amount of the amino acid in this way. This fact is quite significant since it serves to bring out another important point. The formation of monamino acid, by the action of concentrated aqueous ammonia on the halogen substituted acids, never takes place alone as most writers seem to imply. We sometimes have di and triamino acids formed in this action (vide Heintz)[8] and since ammonia acts also as a weak base, as well as an amino compound, we ought to have a large or small amount of hydroxy-

[7] Fisher and Raske: Ber. 40, 1051–7.

[8] Heintz: Ann. 156, 25; Ann. 136, 213–223.

acid always formed simultaneously.[9] These facts may have an important bearing when we test experimentally the second phase of the Walden inversion brought out by Fisher, namely the apparent inversion in the action of nitrosyl bromid on the amino acid and the normal reaction obtained with ammonia on the brom acid to give the corresponding amino acid. In this connection, I may add, however, that the action of nitrosyl bromide on alanin gives a quantitative yield of perfectly homogeneous, constant boiling, brom-propionic acid. If the inversion which seems to occur with nitrosyl bromide, does not take place as a matter of fact, we would simply have to shift the names of our aminopropionic acids, i. e. call laevorotatory alanin d alanin and vice versa, and likewise other homologous amino acids. This would involve no very radical change as we now have just such a condition in the case of the halogen succinic acids—we should have the amino group now and then exerting the same general effect on the absolute rotation of the acid or ester as the substituting halogen group sometimes does. If such is the case, we can test the point most readily experimentally by a reinvestigation of the action of nitrous fumes on dl alanin to determine whether dl lactic acid is really obtained or is the only product formed. It is quite reasonable to believe that nitrous acid may give in this case also a body having all the properties of the acid product which we obtained in the action of moist silver oxide or dl brompropionic acid.

Fisher also stated that the inversion seemed to occur only with α substituted acids i. e. at the asymmetric carbon atom next to the free COOH group and that it took place only with the free acid and never with the ester. By substituting C_2H_5 for H in the COOH group, my own experiments as well as those of others have shown that the reactivity of brompropionic acid toward oxide of silver in absolute ether solution at ordinary temperature is reduced from an extremely high value to practically zero. By thus reducing the speed of the reaction we should naturally expect to get a normal result in the replacement of haloegn by hydroxyl using oxide of silver as substituting

[9] Same as No. 8.

agent. That an inversion should not take place with esters much more reactive than brompropionic, is not at all inconceivable if it really does take place with the free acid; this, however, would supply a serious objection to Fisher's proof of which reagent causes inversion. Later on,[10] Fisher demonstrated that a β halogen substituted fatty acid did not give an inversion with silver oxide and that the action of nitrosyl bromide on the corresponding amino acid was undoubtedly normal in this case also. By moving the halogen one C atom back from the COOH group, the compound ordinarily becomes far less reactive. As a simple ilustration of this, we may compare the action of α chlor and β chlorpropionic acid with silver oxide.[11] By checking the speed of the action, we should expect to get a normal replacement of halogen by hydroxyl, using silver oxide as agent, just as Fisher has determined experimentally with β chlorbutyric acid. McKenzie very recently[12] confirms this observation by proving that phenyl β brompropionic acid does not give an inversion with silver oxide. There is no reason, however, a priori, for not getting an inversion with very reactive β halogen or amino substituted acids as well as α.

The phenomena, giving rise to the 'Walden inversion' (real or apparent), take place, as far as I have been able to judge, as the result of a very rapid action. The replacement of halogen by hydroxyl in a perfectly normal manner takes place when the reaction proceeds more slowly.

We must bear in mind the fact that α brompropionic acid forms sodium and potassium salts which have a fair degree of stability towards water and dilute alkali. I have succeeded in recovering sodium brompropionate almost quantitatively from a water solution of the free acid neutralized with sodium hydroxide and subsequently distiled off at reduced pressure (20 mm) heating finally to 60°C; while, contrary to the statement of Beckurts and Otto,[13] by no conceivable method could I get a trace of

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[10] Ber. [May, 1909], 1219.
[11] Wichelhaus: Ann. 143, 1. Moldenhauer, Ann. 131, 323.
[12] J. L. Ch. Soc. March, 1910, p. 121.
[13] Beckurts & Otto: Ber. 18, 223; Ber. 16, 576. W. H. Perkin; J. L. Ch. Soc. Vol. 11, p. 25; Vol. 32, p. 90.

the silver salt of α brompropionic acid by neutralizing with silver oxide. The reaction between silver oxide and brompropionic acid proceds with tremendous speed once the short period of induction is passed—hydrogen bromide is split off readily and silver bromide separates out quantitatively in a very short time. According to Senter,[14] α brompropionic acid, treated in water solution with silver nitrate, gives a reaction 17000 times faster than that of sodium hydroxide on the same acid. With silver oxide in place of silver nitrate, there is also undoubtedly a vast difference in speed between the two reactions. That we are dealing here with two totally different reactions is the opinio of Senter, Burke and Donnan,[15] Euler[16] and others, who have attacked this problem from the physico-chemical standpoint. That different reaction products are formed remains to be rigidly established.

Fisher and Scheibler's Results and McKenzie's Contribution.

We are now in a position to consider some results, which, when viewed in the right light, may turn out to be a reductio ad absurdum disproof of the whole Walden inversion. In the first place we may ask: Can Fisher's explanation of the Walden inversion be followed out to give a completely harmonious system? No. He has found that in the case of d valin (d α aminoisovalerianic acid) a double inversion occurs i. e. an inversion both with nitrosyl bromide and ammonia, so that as a result of this cycle the original valin is regenerated.[17] In this case we may still believe, as Fisher first thought, that no inversion occurs with either reagent, since Fisher and Scheibler found that bromisovalerianic acid, treated either with potassium hydroxide or with silver oxide,[18] yielded the same hydroxy-acid. Here again we may have, of course, an inversion with both bases—indeed, unless we make such an assumption, the action of nitrous acid must also be considered abnormal, since l valin with nitrous acid gives the same oxy-acid as is obtained by the

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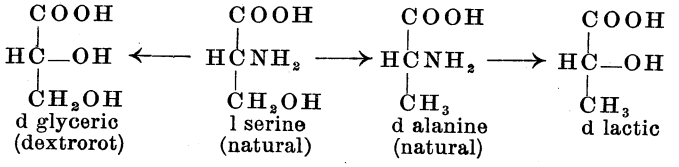
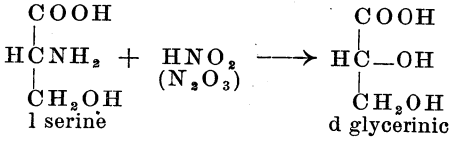
14 Proc. L. Ch. Soc. [1908] 24, 89. J. L. Ch. Soc. Dec., 1909, p. 1827.
15 Trans. L. Ch. Soc. 1904, p. 555.
16 Ber. 39, 2726–2734.
17 Ber. 41, 889.
18 Ber. 41, 2891.

action of potassium hydroxide or silver oxide on d bromisovalerianic acid. These results, when carefully considered, seem to indicate that there is no inversion of optical activity in any of reactions of optically active valin or its corresponding derivatives. Fisher himself is at a loss in considering these reactions for they show that, if there is anything in the idea of a Walden inversion, the influence of any reagent, whether normal or abnormal, can not be predicted, but must be worked out carefully in each individual case. This necessarily means much tedious work in establishing the changes taking place with optically active substances—as an immediate result it tends to throw some doubt on the absolute configuration of the C_3 acids as established by Fisher on the basis of several changes, all of which were assumed to be normal.

NOMENCLATURE REVISED ACCORDING TO NEUBERG'S LATEST WORK.

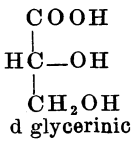


Fisher and Jacobs[19] had shown that d serine had a constitution corresponding to that of d glycerinic, the structure of which had been proved to be similar to that of d tartaric and therefore to d glucose by Neuberg and Silbermann.[20]



l serine gives by treatment with nitrous acid the corresponding hydroxy-acid, (l glycerine acid according to Neuberg and Silbermann) or d glycerinic acid according to Neuberg's latest work (where he shows that d glycerinic acid by addition of prussic acid etc. gives d tartaric acid).[21] d glycerinic acid has the structural formula therefore as given above

[19] Ber. 40, 1057.
[20] Zeit. für physiol. Ch. 44, 134 [1905].
[21] Bioch. Zeit. 5, 451.



d glycerinic

which is in beautiful harmony with the system proposed by Rosanoff.[22] Fisher and Raske succeeded in converting l serine into d alanine,[23] which in turn by treatment with nitrous acid gave d lactic acid.[24] We have, therefore, a rigid demonstration of the configuration of the whole series of the C_3 optically active acids, providing of course, that all the exchanges used in this proof take place normally.

Fisher seems to be about to attack this problem from an entirely different point of view. Making use of Guye and Crum Brown's hypothesis i. e. by simply determining the effect of various substituting groups on the absolute rotation of a large number of organic compounds and taking account also of the relative position of the various groups, one may ultimately be able to calculate the rotation produced by a body of a given configuration. Providing no other side products were formed, we would have complete information regarding the changes taking place with optically active bodies as soon as we had determined the rotation of the product. The exact trend of Fisher's future work on the Walden inversion is hard to determine—his last paper on propyl, isopropyl cyanacetic acid[25] seems to be in the direction indicated here. To attain results of general significance along this line will involve, however, a very considerable amount of tedious work, since the elimination of the effect of the solvent upon the absolute rotation of various substances is a matter of extreme difficulty. Unfortunately, all of our pure organic bodies can not be studied in the form of oils.

We shall now consider some results in another series which may be interpreted on the basis of a double inversion. Shortly after Fisher's first paper was published in March 1907, McKenzie, an English chemist, announced some experimental re-

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[22] J. Am. Ch. Soc. [1906] Vol. 28, p. 118 footnote.
[23] Ber. 40, 3717; Ber. 41, 893.
[24] Ber. 40, 1051.
[25] Ber. [Sept. 1909] 42, 2981–2989.

sults,[26] obtained with optically active phenyl chloracetic acid, which were entirely out of harmony with Fisher's results obtained in the lactic acid series, as well as those of Walden in the malic acid series. McKenzie quickly seized upon Fisher's double inversion idea as a means of explaining some of his apparently anomalous results (i. e. anomalous in the sense of not harmonizing with Fisher's conception of the Walden inversion as proposed in 1907). He found that optically active mandelic acid, treated with phosphorous pentachloride and then with potassium hydroxide, yielded its antipode while successive treatment with phosphorous pentachloride and oxide of silver gave back the same optically active mandelic acid as was used at the start. To obtain a harmonious explanation of these results, McKenzie, in his second article,[27] held that the action of phosphorous pentachloride might be considered as abnormal in this case; the action of potassium hydroxide would then be normal and that of silver oxide abnormal just as Fisher and Walden found in other series. But this assumption (that phosphorour pentachloride may act abnormally) did not harmonize with the following observations by the same author.

d mandelic + PCl_5 ⟶ laevorotatory d phenyl chloracetic
ethyl d mandelate + PCl_5 ⟶ laevorotatory d phenyl chloracetic ethyl ester.

He was forced to interpret, therefore, that silver carbonate acted *normally,* just as it may in Fisher's experiment with optically active valin, and that the action of potassium hydroxide was *abnormal.* These results are exactly the opposite of those obtained by Fisher and Walden in the lactic and malic acid series respectively. McKenzie found also that the action of water was *abnormal* comparable to the action of strong bases on halogen acids of this series while Walden found that the action of water was similar to that of silver oxide and other weak bases. When phenylamino acetic acid was treated with nitrosyl bromide and the resulting brom acid treated with ammonia, the original amino acid was recovered. McKenzie explained this

[26] J. L. Ch. Soc. [May 1908] Vol. 93, p. 81.
[27] J. L. Ch. Soc., May 1909, p. 777.

result on the basis of an inversion with both reagents entirely analagous to the double inversion which Fisher seems to have rigidly established in the case of optically active valin. There is very little danger that McKenzie will contribute anything startling to our present knowledge of the Walden inversion' as he is unquestionably simply following Fisher's lead in another series. As a serious objection to McKenzie's work he has yet to prove, as far as I can see, that his phenyl chloracetic acid, when treated and allowed to stand with solutions of various dilute alkalies in cold water,[28] even by boiling finally for a short time (30 to 60 minutes) actually splits to any considerable extent, or, if it does split completely, that he obtains quantitatively mandelic acid. If very little splitting actually takes place, then McKenzie's results for stronger alkalies in water solution are valueless while oxide of silver, at all events, may be very reasonably held to give a normal substitution product—a result at variance with the facts established by Fisher, Walden and others who have used this reagent. Inasmuch as mandelic acid is a beautiful crystalline body, these crucial experiments, when carefully repeated, ought to give much more definite results than are obtained with malic and lactic acid since both of the latter give oily derivatives difficult to identify sharply. Of course it must be admitted that the introduction of the phenyl group exerts a powerful influence, but it may be said in this connection that monochloracetic acid gives sodium and potassium salts which are very stable in water and alkaline solution[29] while the halogen, being in the α position, is very easily removed with silver oxide.

As I have tried to point out, future progress in disentangling the 'Walden inversion' will depend not on establishing more analogous inversions in other series, for this will simply add to the confusion already existing.

A rational explanation for the results obtained, where an inversion of optical activity appears to take place, will undoubt-

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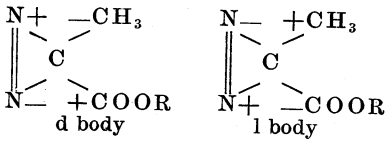
[28] J. L. Ch. Soc., May 1909, p. 782 et seq. Expt. part.

[29] Senter: J. L. Ch. Soc. Sec. 1909, 1827; Hoffman: Ann. 102, 6; Kekule: Ann. 105, 288.

edly be found as a result of most careful experiments to establish the changes which actually occur in the action of various reagents on optically active bodies, all of which have been assumed to take place as a result of a simple metalepsis. Scarcely any of these changes have been rigidly established. Almost without exception,[30] satisfactory figures for solubility, water of hydration and percentage of zinc have sufficed to prove the presence of lactic acid in the form of its zinc salt. When the reader weighs carefully the evidence presented in the experimental part of this paper, he will undoubtedly agree with me that the Walden inversion simply represents another case of some peculiar co-incident results which have often led scientists astray.

Some recent experimental results.

This investigation was undertaken at the suggestion of Prof. J. U. Nef of the University of Chicago with the purpose of obtaining independent experimental evidence to support his idea that the four valences of the carbon atom are not mutually equivalent, but only in pairs. Acording to this idea we ought to be able to prepare two space isomeric diazo propionic esters resp. d and l.



d body l body

The preparation of diazo fatty esters is no easy matter and besides it was necessary first to become thoroughly familiar with the properties of the optically active acids of the C_3 series.

Optically active alanin was prepared according to Fisher's method by resolution of benzoyl alanin by means of brucin in

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[30] Wislicenus: Ann. 166, 6; Heintz: Ann. 156, 25; Ann. 157, 295; Wichelhaus: Ann. 143, 1; Friedel & Machuca, Comptes Rendus, 13, 408; Buff: Ann. 140, 156; E. Fisher; Ber. 40, 1051.

[30] E. Fisher & A. Skita: Zeit. fur physiol. Ch. [1901] Vol. 33, 190; E. Fisher & Zemplen: Ber. Dec. 1909, 42, 4878–4892; Beckurts & Otto: Ber. 18, 222–238.

aqueous solution. The laevorotory alanin, thus obtained, was converted by means of nitrosyl bromide quantitatively into dextrorotatory d brompropionic acid according to the latest method of Fisher and Raske. The active brom acid, as prepared, contained 4.38% of optical antipode as against 3% usually found by Fisher.

Inasmuch as nearly all of the experiments, where an inversion was observed, had been carried out in water solution, we attempted to ascertain whether the same inversion occurred in non-aqueous solution in order to establish, first of all, the influence of the solvent. To avoid also the presence of any possible trace of free base, Prof. Nef proposed the idea of studying the action of silver acetate (1 mol) on d brompropionic acid in absolute ether solution.

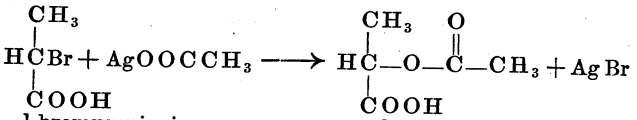
By hydrolysis of the crude (acylated?) gum thus obtained, and subsequent heating in water solution with zinc carbonate, a difficulty soluble crystalline zinc salt was obtained having all the properties of zinc lactate. Most of this material was inactive—silver acetate seems to have, therefore, a pronounced racemizing effect just as Marckwald and Nolda have found in its action on optically active amyl haloids at higher temperatures. The mother liquor contained a considerable amount of zinc salt which was strongly dextrotatory, from this fact we may assume, as others have done, that there is formed here a little l lactic acid, mixed with a large amount of dl lactic i. e. an inversion has occurred with silver acetate in absolute ether as well as with silver salts in water solution. The solvent undoubtedly causes a difference in the amount of racemation but does not alter the character of the action as far as inversion is concerned.

By the action of silver acetate (1 mol.) in water solution, potassium acetate (1 mol.) in absolute alcohol solution, and sodium hydroxide (1 mol.) in 0.1% water solution on d brompropionic acid there was obtained after hydrolysis in every case, on treatment with zinc carbonate, a difficulty soluble crystalline zinc salt which was more strongly dextrorotatory than that obtained in the experiment with silver acetate in absolute ether.

In accordance with the usual interpretation that nothing but lactic acid is obtained here as ultimate product, we have here in each of these three cases an inversion of optical activity. These experiments serve to disprove Walden's idea that the inversion takes place only with silver hydroxide, water, and analagous weak bases and never with bases or salts derived from such metals as sodium, potassium, etc., whose action on water gives rise to the strong bases. As a result, we see that the inversion may occur in any solvent, aqueous or non-aqueous, and is caused by comparable very slight concentrations of all bases, and of those salts which give bases by dissociation or hydrolysis. That an inversion of optical activity really takes place in any case, in the action of weak bases on halogen substituted fatty acids, becomes decidedly open to question when we consider the folowing data obtained from experiments with dl (inactive) brompropionic acid.

Space will not permit me to give, as I should like, a detailed description of all the experiments which I have carried out in order to determine the changes taking place in the action of silver oxide and silver salts on d and dl brompropionic acid. I shall content myself with the following summary of results with dl mat.:

In the action of silver acetate on d, l or dl brompropionic acid in absolute ether we should expect to get by double decomposition, complete transformation to α acetyl hydroxy-propionic acid (acylated lactic) soluble in water of b. pt. 134° (15 mm.).



l brompropionic — l acetyl hydroxy propionic acid.

We get, however, as main product a stiff gum insoluble in water. This gum by hydrolysis with water should give lactic acid of it consisted of oily lactid or poly lactyl lactic acid. By hydrolysis and distillation of the water at reduced pressure, I obtained, to my surprise, in place of mobile, syrupy lactic acid titrating over 90% in the cold, a gum, soluble in water, which had but very little mobility and which, with N/10 potassium hydroxide, titrated only 50% as a free acid. This

hydrolyzed product therefore had all the properties of the acid gum obtained by hydrolysis of the crude product of the action of silver acetate on d brompropionic acid in absolute ether.

Silver oxide, as well as silver carbonate, in water solution yielded a gum which was soluble in water and titrated for the most part as a free acid. By heating, however, with water at 120° for 6 hrs. in oil bath, there was obtained, after distilling off water at reduced pressure, a stiff gum which was soluble in water and titrated only 50% as a free acid. Even after dissolving in excess of 5% soda solution, and heating alkaline solution 5–6 hrs. at 100° the gum recovered by neutralization with stand. dil. HCl, distilling off water at reduced pressure etc., was soluble in water but had very little mobility and titrated only 50–60% as a free acid. *Can heating in water solution at 100° in the presence of an excess of zinc carbonate convert this material into zinc lactate, where heating with water at 120°, or with an excess of sodium carbonate at 100° did not yield lactic acid or sodium lactate resp.?*

Acrylic acid or hydracrylic acid might easily be obtained here.

$$CH_3\overset{H}{\underset{Br}{C}}—COOH + Ag\,OH \longrightarrow CH_2 = CH\,COOH + Ag\,Br$$

If the acrylic acid then in part added water again we should get hydracrylic acid (β hydroxy propionic).

$$CH_2 = CH — COOH + HOH \longrightarrow CH_2\,OH — CH_2\,COOH.$$

That neither of these is present is shown by the fact that the zinc salt crystallizes almost completely from water due to its difficult solubility, while the zinc salts of acrylic and hydracrylic acids are readily soluble in water.

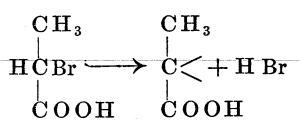
Dilactic acid might be formed by simple metalepsis:

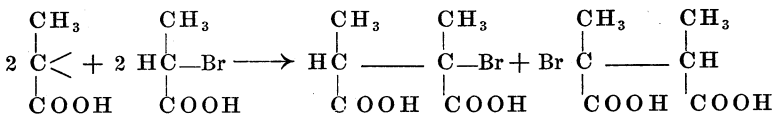
$$CH_3 \overset{H}{\underset{Ag\quad O\quad Br}{\text{——}C}} COOH$$

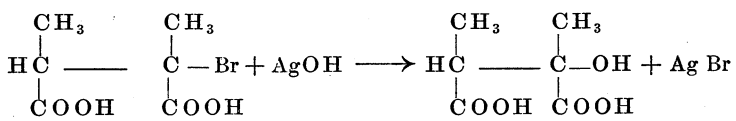
$$CH_3 \overset{Ag\quad Br}{\underset{H}{\text{——}C}} — COOH$$

That dilactic acid is not present is shown by the fact that the crude acid product obtained by the action of silver oxide on dl brompropionic, although proved to be homogeneous, shows no tendency to crystallize. In addition brucin dilactate was found to have dec. pt. 110°–138° while the brucin salt of the crude acid gum melts 200°–212° very similar to that of brucin lactate, and other high melting brucin salts.

From the standpoint of dissociation according to Nef, carboxyethylidene is formed first as intermediate product. By subsequent addition of unchanged brompropionic acid we should expect as the ultimate product *not dilactic acid but its structural isomer* which exists in two space isomeric modifications:







Work is now being continued along this line by the author of this article in the chemical laboratory of Beloit college. An attempt will be made to prepare this body synthetically and study its properties. From its structure we should expect it to behave very similar to lactic or dilactic acid and in addition it ought to give very readily a 1, 2 lactone.

In conclusion, I will say that, by using absolute ether in place of water as solvent, one is enabled to study the action of silver oxide on the simple esters of the halogen substituted fatty acids without danger of hydrolysis. In marked contrast to the behavior of the free acid, dl brompropionic ester does not react at room temp. with silver oxide in absolute ether solution. By

heating dl brompropionic ethylester with silver oxide several hours at 120° the main product in the action was dilactic diethyl ester just as in the action of the sodium salt of lactic ethyl ester on dl brom propionic ester, acording to von Bruggen.

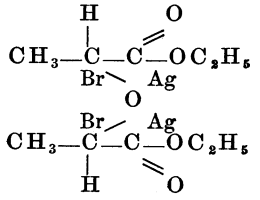


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Table of Abbreviations used in preceding footnotes and in this bibliography.

(1) Ann.=Liebig's Annalen der Chemie und der Pharmacie.
(2) Ber.=Berichte der Deutschen Chemischen Gesellschaft.
(3) C. R.=Comptes Rendus de l'Academie.
(4) Ann. de Ch. et de Phys.=Annales de Chemie et de Physique.
(5) J. Ch. Soc. or J. L. Ch. Soc.=Journal London Chemical Society.
(6) J. Am. Ch. Soc.=Journal American Chemical Society.
(7) Am. Ch. J.=American Chemical Journal.
(8) Bioch. Zeit.=Biochemische Zeitschrift.
(9) Zeit. für phys. Ch.=Zeitschrift für Physikalische Chemie.
(10) Zeit für physiol. Ch.=Zeitschrift für physiologische Chemie.
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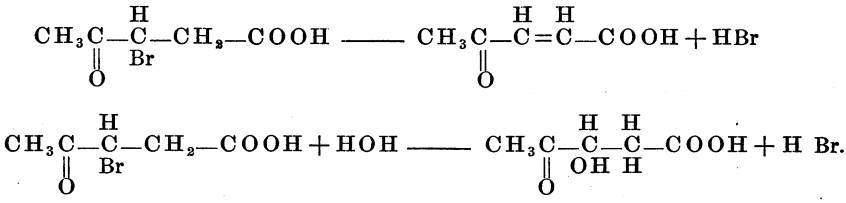

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THE CHEMISTRY OF BORON AND SOME NEW ORGANIC—BORON COMPOUNDS.

ARDEN R. JOHNSON.

While engaged in carrying on a detailed review of the chemistry of several individual elements, among which was boron, I was greatly impressed by the meagre literature on the chemistry of this element. There are but very few compounds, artificially prepared, described; and those which dame nature furnishes us are exceedingly rare, with two or three exceptions, and appear to play very slight rôles in the economy of the world, either mineral or biological. Very likely it is this apparent insignificance in nature and lack of any immediate important, industrial applications of boron and its compounds that has given rise to its neglect by chemists. It was to satisfy my curiosity as to whether this element offers a wide and virgin field of new, synthetic products of curious properties, or presents unsurmountable difficulties which have really baffled chemists, that moved me to attempt some preliminary work during the summer of 1909 at the University of Wisconsin.

If we study boron in the light of the periodic system we find that it occupies a position between metals and non-metals, and also near carbon and silicon, which elements are also near the border-line. We indeed find a strong similarity between boron and these two elements, especially in physical properties, e. g. Amorphous boron is much like amorphous carbon, and has a chestnut-brown color. The corresponding graphitic and diamond boron are also known. However, in most of its com-

pounds boron is trivalent instead of tetravalent as is the case with carbon and silicon compounds. In the latter property boron is more like the members of the nitrogen group, in fact there is some evidence that it may act as a pentavalent element in certain compounds. Theoretically, boron should act very much like aluminum also. Notwithstanding, excepting the oxide B_2O_3, there are not so very many close analogies. In fact there is a very decided similarity to the tri- and pentavalent elements, nitrogen and phosphorous, in chemical behavior, although they are decidedly non-metallic elements. And strange enough, though boron must be regarded as more of a non-metal than a metal yet it reacts far more readily with non-metals than metals; borides of metals are very difficult to prepare.

Among the very few inorganic compounds of importance are the halogen borides. These were merely prepared by several workers but not studied to any extent. Their chemical comportment toward other reagents has remained a virgin field. $B Cl_3$* is a liquid boiling at 20°; $B Br_3$ boils at 91°; and $B I_3$ boils at 210°. The latter compound is decomposed quite readily by light. $B Br_3$ on account of its stability and high boiling point has been taken as starting material for the synthesis of boron compounds in my work. The action of all the halogen compounds, which are being prepared from time to time by the combustion method as needed in my work, toward typical organic and inorganic compounds, and their physico-chemical properties, as indexes of refraction, latent heat of vaporization, etc., are being studied and will soon be reported on in a separate paper.

In studying the chemical comportment of the halogen borides, the tetrahalogen compounds of carbon and silicon on one hand, of phosphorus and nitrogen on the other, and also of aluminum have been kept steadily in mind. That is for practical purposes, I regard boron as occupying the center of a triangular field bounded by the above mentioned groups of elements and aluminum.

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* Nickles—C. R. 60-800-1865; Moissan—C. R. 112-717-1891; Besson—C. R. 113-78-1891.

A most interesting matter, both from the theoretical standpoint and the properties of the products, is the fact that boron compounds like BBr_3, for example, form a remarkable number of so-called addition products with compounds of the kindred elements on the three sides of the triangular field occupied by boron. Some of these compounds are: $2BBr_3$, PCl_3; $2BBr_3$, PCl_5; BBr_3, PH_3; BBr_3, PBr_3; BBr_3, $4NH_3$; but $AsBr_3$ and $SbCl_3$ merely dissolve in BBr_3, no definite compounds separate. At the present time I am interested in the preparation of compounds of aluminum halides and boron halides. $AlCl_3$ and BBr_3 appear to give a definite compound, but as yet I have not completed an analysis of it. However, it appears to break up under high temperature and in hydrogen atmosphere to a boride of aluminum.

Of the compounds mentioned that with ammonia is of most interest to me theoretically. Indeed, it was the study of this compound which lead me to attempt to prepare some new organo-boron compounds by starting with BBr_3 and members of those classes of organic compounds characterized by the presence of an NH_2 group, or the NH group (imido), or N only as in the case of pydrine, quinoline and others. Thus far my work has been principally upon amines and pyridine.

If solutions of BBr_3 and aniline in carbon tetrachloride, carbon bisulphide or benzine are brought together drop by drop an insoluble, amphorous precipitate appears. The action is accompanied by great evolution of heat. When the precipitate is carefully dried in a nitrogen or hydrogen atmosphere and analyzed it is found to be $2BBr_3$, $C_6H_5NH_2$. With an excess of aniline and higher temperature of reacting system there results a yellow substance analyzing up to $2BBr_3$, $3C_6H_5NH_2$. The first decomposes water quite violently, while the latter acts slowly on water giving $B(OH)_3$ and aniline hydrobromide. If the $2BBr_3$, $3C_6H_5NH_2$ is allowed to stand in a strong light in an air tight dessicator in which there is some strong alkali in a separate dish, HBr is evolved from the substance and there remains what analyses up to a sort of boro-nitride of anniline. When the product is placed in water but very slight decompo-

sition occurs, even in several days. But if the water is heated action soon sets in and continues quite rapidly giving aniline and boric acid.

Pyridine and BBr_3 form a white, amphorous precipitate in CCl_4 as medium. It is surprisingly stable in water, not decomposing readily unless boiled for some time. It analyses up to BBr_3, C_5H_5N.

The methods of analysis relied upon in the foregoing work consisted in determining the bromine gravimetrically as silver bromide, and the nitrogen by the Kjeldahl procedure. The combustion method will be worked later but thus far the Kjeldahl method has given sufficiently satisfactory results.

Another phase of boron chemistry which presents interesting aspects is the action of born halides on the unsaturated hydrocarbons. The olefine and acetylene series. As an example: Dilute solutions of amylene and BBr_3 in CCl_4 as a medium were brought together slowly, the system being cooled with ice-water, and to my surprise, pure, amorphous boron was precipitated in an extremely attenuated, colloidal condition. The boron will, in fact, remain suspended in the reaction mixture an indefinite time. That the material was pure boron, was proved beyond a doubt by evaporating off all the volatile products and washing the residue with carbon tetrachloride and then with ether. As a film it was of a maroon color, very stable in air, even when heated to about 500°. When heated in a glass tube with pure oxygen it was burned to white boric oxide. The gas drawn from the tube and then through lime-water gave no test for carbon dioxide.

The reaction of BBr_3 and the unsaturated compounds is doubtless very complicated, however, and very exact conditions must be maintained to obtain certain definite end-products. If BBr_3 and amylene are brought together directly they react with explosive violence and there is charring of the organic substance, and most irritating fumes evolved. As the other extreme, the passage of BBr_3 fumes carried by a current of hydrogen into amylene dissolved to the extent of 10 to 20 per cent in carbon tetrachloride and the whole reacting mass cooled

in ice water, gives rise to no precipitate but to a most disagreeable smelling solution from which I have not yet succeeded in isolating a definite compound.

As soon as the above work is carried out in detail in several of its many ramifications I expect to study the action of boron halides on aldehydes, ketones and other typical classes of organic compounds and compare the results with those usually obtained with free halogens or the halides of phosphorous and aluminum.

A STUDY OF THE LIGHT REACTIONS OF PILOBOLUS.

RUTH F. ALLEN AND HALLY D. M. JOLIVETTE

INTRODUCTION.

The study of the reactions of plants to simultaneous stimuli, either of the same or of different kinds, affords perhaps one of the best means of approach to the fundamental questions of the physiology of stimulation of living organisms. The simpler organisms, devoid of specially differentiated parts for receiving and transmitting stimuli, are especially favorable for the study of such problems; and plants, because of their relative slowness of response, afford good opportunities for measuring and analyzing the phases and elements of their reactions.

As is well known, *Pilobolus* is strongly heliotropic and fires its sporanges toward an illuminated spot. By catching the sporanges on an interposed glass plate as they are discharged, we have a simple means of obtaining mathematical data as to the nature and accuracy of the reaction. Furthermore such difficulties of interpretation as are found in dealing with the reactions of the complex and highly differentiated growing tissues of higher plants are eliminated here, for we are concerned with the reactions of practically a single cell.

In the preliminary studies here described, we undertook to obtain data which might assist in answering the following questions: (1) How accurately will *Pilobolus* fire its sporanges toward a source of light? (2) Does *Pilobolus* direct its sporanges straight toward a source of light, or does it aim high according to the distance at which it is placed from the source of illumination? (3) In what direction will *Pilobolus* discharge

its sporanges when it is presented simultaneously with two sources of light of equal intensity? (4) What is the relative efficiency of light of different colors presented successively or simultaneously in determining the direction in which the sporanges will be fired?

As early as 1874, Tode (22) discovered that *Pilobolus* discharged its sporanges with considerable violence toward a source of light.

The reactions of fungi to light of different colors were studied by Sorokin (20). Cultures were grown on dung under double-walled bell jars in white, blue, and yellow light, and in the dark. He found that *Mucor* and *Pilobolus* show positive heliotropism in white light, and negative heliotropism in yellow light. The spores of *Pilobolus* are seldom scattered in the yellow light. In *Pilobolus,* positive heliotropism is manifested through the firing of the sporange toward the light in question. In the dark, the hyphæ of both genera grow upright and become much elongated.

Fischer von Waldheim (9) studied the light reactions of *Pilobolus* by the same methods and arrived at different results. In cultures kept in the dark, the sporangiophores stood out at right angles to the surface of the substratum, no matter whether the surface was horizontal or vertical. The organism behaved the same in yellow light, no negative reaction being observed.

Brefeld (4), using the same methods, finds that sporangiophores of *Pilobolus microsporus* are strongly positively heliotropic in yellow light, although the sporanges never mature. By turning around every few hours a culture illuminated from one side, the sporangiophore bends successively back and forth and becomes zigzag in form. He concludes from this that the zone of growth is at or near the apex.

Regel (19) also studied the bending reactions of *Pilobolus crystallinus* as well as those of *Mucor mucedo.* According to him, these molds are positively heliotropic in white, blue, yellow, and red light. This is true for all light intensities and temperatures tried. The more refrangible rays are more effi-

cient in causing heliotropic curvatures than are the less refrangible.

Noll (18) used in studying the light reactions of *Pilobolus* a box with an opening at one end through which daylight could enter. Inside the box, be placed a culture with the surface sloping toward the opening. According to Noll, the grouping of the sporanges about the opening after their discharge showed that they were directed toward the center of the opening. His schematic figure of the sporangiophores just before being discharged shows that those on the part of the culture furthest from the opening were pointed far above the opening. He, however, says nothing about this in his description. No statement is made to show whether or not he believes that the sporangiophores have aimed high in order that the sporanges may not fall below the opening. Noll believes that the final position of the sporangiophore is not a resultant of a heliotropic and of a geotropic reaction, but that it is due to light alone.

Oltmanns (16) describes experiments with *Phycomyces* to determine whether there is an optimum light intensity for its reactions. For these experiments he used a box one meter in length and twenty-five centimeters in breadth and height. He placed an arc light before the opening at one end of the box, and intercepted the heat rays by placing a flask of running water between the light and the box. The cultures were placed at ten centimeter intervals along the median line of the box. A negative reaction was shown by those at from twenty to thirty centimeters distance, and a positive reaction by those at from seventy-five to eighty centimeters distance. Those between these extremes were straight. This intermediate region, according to Oltmanns, is the region of optimum light intensity. Somewhat later, the region in which the sporangiophores did not bend was between sixty and seventy centimeters. Still later it receded to from fifty to sixty centimeters from the light. Oltmanns concludes that the more accustomed the sporangiophores become to the light the higher is the optimum light intensity; that is, the nearer to the light will be the region where the sporangiophores remain straight. He also concludes that

in the region of optimum illumination the vertical position of the sporangiophore is due to geotropism.

Graenitz (10) repeated Brefeld's experiments on *Pilobolus* and *Coprinus* and corroborated his results.

Steyer (21), like Oltmanns, found that *Phycomyces* is negatively or positively heliotropic according to the intensity of the light used, and that plants grown in the light are less sensitive than those grown in the dark. He determined the region of the sporangiophore which was sensitive to light by allowing a horizontal slit of light to strike the sporangiophore at different places. He found that the zone of growth (the portion of the sporangiophore just beneath the sporange) alone is the sensitive region, and that the rapidity of the reaction is dependent on the rate of growth. Steyer showed further that during artificial inhibition of growth by ether, the sporangiophore is in a position to perceive a light stimulus. He exposed the fungus to light while it was under the influence of ether. No visible reaction took place. The culture was then placed in the dark and the effects of the ether were allowed to disappear. The plant then reacted to the light stimulus which it received when under the influence of ether.

The more recent literature dealing with the physiological efficiency of various colors, the effects of simultaneous stimulation, and related topics will be taken up below in the discussion of our own results.

Methods

In our experiments we used cultures of *Pilobolus* grown in two-inch flower pots. The experiments were made in light-proof boxes painted black inside and provided with one or more openings for admitting light. A movable pane of glass, cut to fit the inside dimensions of the box, was placed either against the end containing the opening or parallel to it and nearer the culture. Sporanges discharged from the culture toward the source of light were intercepted at any desired distance by the glass plate and remained attached to it. The sporanges could easily be seen with the naked eye. From cultures of the size

used, fifty to five hundred sporanges are fired each day. The glass plate was removed from the box daily and laid upon a sheet of paper upon which a target was drawn, the form and dimensions of which will be described in connection with the respective experiments, and an exact record was made of the number and position of the sporanges upon that portion of the glass plate which corresponded to each area of the target.

As is well known, there is a daily periodicity in the formation of the sporanges of *Pilobolus,* the ripe sporanges being regularly discharged in the morning. The plate on which the sporanges struck was removed in the late afternoon or evening. The sporanges had then been all discharged and the next day's crop was beginning its development. One culture generally produced three or four crops sufficiently abundant for use; occasional cultures produced a large number of sporanges daily for more than a week. In the case of series of observations extending over longer periods than these, new cultures were used to take the place of those exhausted. The cultures were watered daily at the time when the plates were removed. For this purpose, the cultures were generally taken out of the box and then replaced. This was sometimes done late enough in the day so that the sporangiophores were sufficiently developed to have taken a definite position with reference to the light. In such cases the culture was replaced as nearly as possible in its original position. In spite of the utmost care, a certain amount of readjustment to the light on the part of the sporangiophores was doubtless sometimes necessitated. Experiments to be described below, however, showed that the final results of the experiments were in no way affected by the necessity of such a slight re-aiming.

The Response of Pilobolus to the Stimulus of a Single White Light.

As a preliminary to the study of the effect of exposure to simultaneous stimuli, several series of experiments were made in order to learn how accurately the sporanges of *Pilobolus* are discharged toward a source of light, using various colored lights

with openings of different sizes and with the cultures at varying distances from the source of illumination. We first attempted to determine the influence of the size of the opening upon the accuracy of aim.

The first experiment was made with white light admitted through a circular opening one centimeter in diameter. An apparatus was used similar to that described by Noll (17). A dark box 121 centimeters long, 27 centimeters wide and 28 centimeters high was used. The opening for the light was in one end. The box was placed with this end facing toward, and about 30 centimeters from, a south window. Direct sunlight did not strike the cultures. Within the box the well-watered culture, five centimeters in diameter, was so placed and supported that its surface was vertical, squarely facing the open-

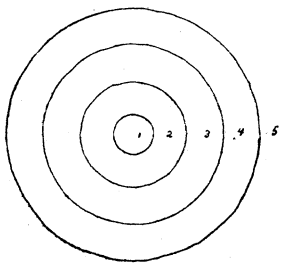

Fig. 1. Target consisting of four concentric circles. The inner circle is 1 cm. in diameter. The circles are 1 cm. apart.

ing, at a distance of 20 centimeters, and with the center of the culture on a level with the center of the opening. A glass on which the sporanges were to be caught was placed inside the box directly in front of and against the opening.

The target used in recording the position and relative distribution of the sporanges consisted of four concentric circles, the radius of each being greater by one centimeter than that of the next circle within. The innermost circle (the bull's eye), one centimeter in diameter, was of the same size as the opening in the box. The areas marked off by the circles were numbered consecutively from one to five, beginning with the bull's eye (Fig. 1). Area 5 included all outside of area 4. By means of this target the percentage of the sporanges which landed upon the illuminated opening and within definite limits out-

side could be determined. In order to count the sporanges in each area, the glass on which the sporanges were caught was placed on a paper target with the areas marked off as noted above so that the part of the glass which stood opposite the opening coincided with the bull's eye on the target.

Only one experiment of this sort with an opening one centimeter in diameter was made with white light. The number of sporanges discharged was 222. Of these, 90 (40.5%) struck the glass opposite the opening, within area 1. 94 (42.3%)

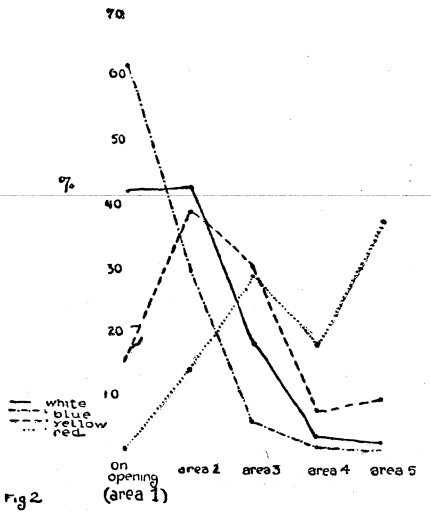

Fig. 2.

were found on area 2, 25 (11.2%) on area 3, 7 (3.1%) on area 4, and 6 (2.7 %) on area 5. A curve plotted from these data is shown in Figure 2 (continuous line), using the percentages as ordinates and the areas as abscissas.

It is seen that nearly eighty-three per cent of the total are found in areas 1 and 2, but of this number a few more are on area 2 than on area 1. It is to be remembered that area 1 is very small as compared with area 2, and also as compared with the size of the culture, which is five centimeters in diameter. On the whole, the data show that the sporangiophores are not able to aim with a great deal of accuracy at a one centimeter opening at 20 centimeters distance. Whether the results would be the same if the same number of single plants were each placed

opposite the center of the opening is of course not shown by this experiment.

In the next experiment, the light was admitted through an opening four centimeters in diameter, the culture still being kept at 20 centimeters distance. The target (Fig. 3) consisted of three concentric circles one centimeter apart. The inner circle was two centimeters in diameter. Consequently, areas 1 and 2 are both within the illuminated area. The size of the inner circle was chosen with the possibility in mind that the sporanges might be fired toward the center of the illuminated area. The areas were numbered from within outward as before, area 4 being all the space outside the third circle.

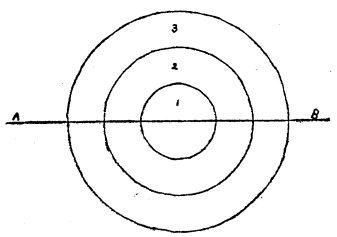

Fig. 3. Target consisting of three concentric circles 01 cm. apart. The inner circle is 2 cm. in diameter. Line A B is a horizontal line drawn through the center of the circles.

In this experiment the total number of sporanges discharged was 93. 67 (72 %) of this number were found on area 1; 21 (22.5%) on area 2; 2 (2.1%) on area 3; and 3 (3.2%) on area 4. The curve plotted as in the foregoing experiment is shown in Figure 4 (continuous line). 94% of all the sporanges landed within the area covered by the four centimeter opening—more than twice the proportion that landed within the area of the smaller opening in the preceding experiment.

The next series of experiments was intended to test the success of the sporanges in striking an illuminated area when the culture is removed to successively greater and greater distances in a horizontal direction. The object was to show whether the organism compensates for the effect of gravity on the path of the sporange by aiming high at a more distant source. The lower edge of the culture was kept on a level with the lower edge of the opening. Since the surface of the culture was five

centimeters in diameter and that of the opening but four centimeters this brought the upper edge of the culture one centimeter above the upper edge of the opening. A series of daily records was obtained by moving the culture back from the opening along the median line of the box so that the distance would be increased two centimeters at a time, allowing it to stand twenty-four hours in each position. The glass on which the sporanges were caught was placed inside the box against the

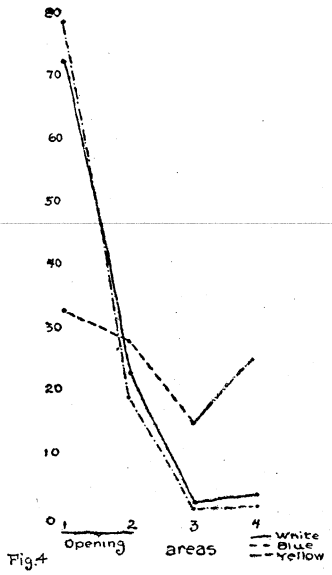

Fig. 4.

opening as before. When the distance of the culture from the opening became so great that some of the sporanges did not reach the glass, a second pane of glass was placed on the bottom of the box to catch them.

In these experiments, the bull's eye target (Fig. 3) described in the last experiment was employed, with the addition of a horizontal line *A B* through the common center of the concentric circles. By means of this line *A B* it was possible to determine whether or not the center of distribution of the sporanges on the glass is lowered as the distance of the culture from the opening is increased. The line *A B,* however, was not used in making the records in this set until the culture had been

moved back to a distance of 50 centimeters. The culture was moved back 2 centimeters each day from 2 centimeters to a final distance of 94 centimeters from the opening.

The first day, with the culture at 2 centimeters from the opening, 255 sporanges were found on the glass. 50 of these (19.6%) were on area 1, 201 (78.8%) were on area 2, making 98% on the area covered by the opening. Only 4 (1.5%) were on area 3 and none at all on area 4.

At 4 centimeters distance from the opening, 130 sporanges were discharged. 71 (54.6%) were on area 1, 49 (37.6%) on area 2, and 5 (3.8%) on each of the two areas 3 and 4. In this case, 92.2% were on the area covered by the opening. 7.6% fell beyond the opening.

The total number of sporanges counted on the glass when the culture was at 6 centimeters distance was 301. 135 of these (44.8%) were found on area 1, 156 (51.8%) on area 2, (0.9%) on area 3, 7 (1.6%) on area 4. In this case, 96.3% were on the part of the glass covering the opening and less than 5% failed to strike the illuminated area.

With the culture at 12 centimeters distance, 104 sporanges were found on the glass. Of these, 61.5% were on area 1; 30.7% on area 2; 6.7% on area 3, and 0.9% on area 4. There were then 92.2% on the opening, the percentage of sporanges outside the illuminated area being 7.6.

At 26 centimeters distance a weak culture which produced only 43 sporanges was used, 65.1% falling on area 1; 23.2% on area 2; 4.6% on area 3, and 6.9% on area 4. 88.3 per cent are on the area covering the opening. 11.5% are found outside of that area.

With the culture at 36 centimeters distance, the total number of sporanges discharged was 218. 22.4% of this number were outside the illuminated area.

With the culture at 48 centimeters distance, 99 sporanges were discharged on the glass. Of these, 54.5% were outside the area covering the opening. As the culture was moved still further back, the number diminished, until at 70 centimeters distance only 1.1% were on areas 1 and 2. At 94 centimeters

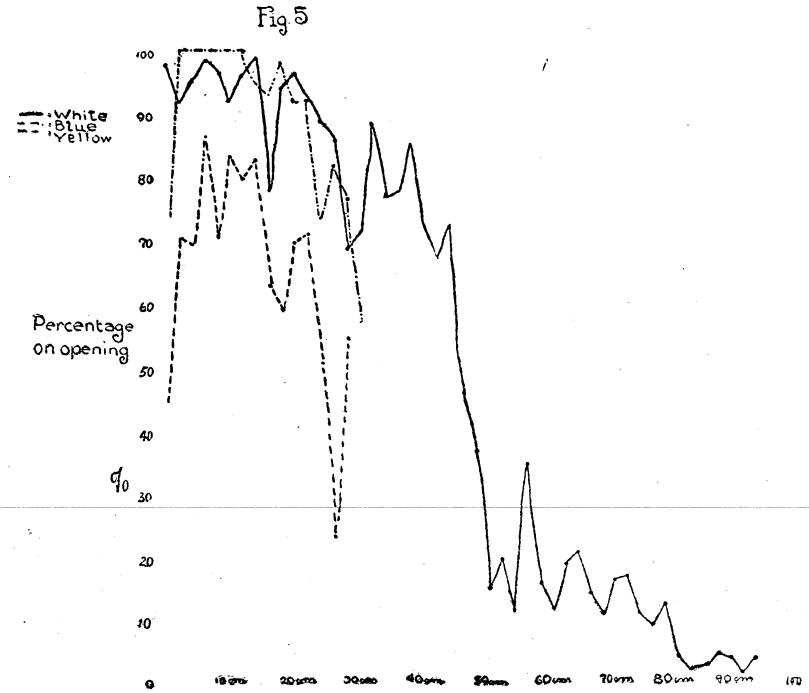

Fig. 5.

distance no sporanges at all were found on area 1 and only 2.4% on area 2. In this case almost all of the sporanges were on area 4. The results of these experiments are shown more in detail in Table I, and graphically by the continuous curves in Figures 5, 6, and 7.

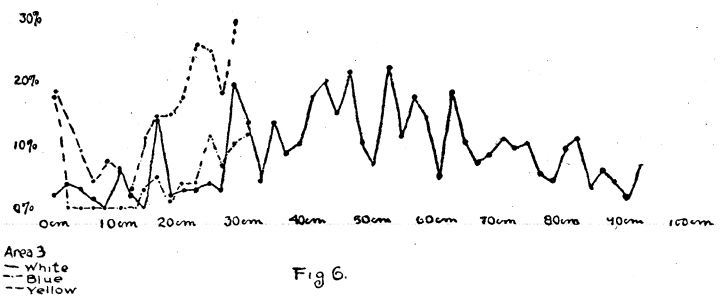

Fig. 6.

TABLE I.—*Cultures at 2-94 cm. Opening 4 cm. in. diameter—white light.*

	2 cm.		4 cm.		6 cm.		8 cm.		10 cm.		12 cm.		14 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
1	50	19.6	71	56.	135	44.5	47	67.7	9	28.1	64	61.5	162	75.
2	201	78.8	49	37.6	156	51.8	103	30.9	22	68.7	32	30.7	45	20.8
3	4	1.5	5	3.8	8	2.6	2	1.3	0	0.	7	6.7	5	2.3
4	0	.0	5	3.8	7	2.3	0	0.	1	3.2	1	.9	4	1.8
Total.....	255	100—	130	100—	301	100—	152	100—	32	100—	104	100—	216	100—

	16 cm.		18 cm.		20 cm.		22 cm.		24 cm.		26 cm.		28 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
1	70	61.9	31	48.4	67	72.	63	81.	99	81.1	28	65.1	33	53.2
2	42	37.1	19	29.6	21	22.6	13	16.6	15	12.2	10	23.2	20	32.2
3	0	0.	9	14.	2	2.1	2	2.5	3	2.4	2	4.6	2	3.2
4	1	.8	4	6.2	3	3.2	0	0.	5	4.	3	6.9	7	11.2
Total.....	113	100—	64	100—	93	100—	78	100—	122	100—	43	100—	62	100—

	30 cm.		32 cm.		34 cm.		36 cm.		38 cm.		40 cm.		42 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
1	21	46.6	22	30.5	264	55.9	91	41.2	42	34.1	42	28.	54	23.2
2	10	22.2	30	41.6	165	34.9	78	35.7	54	43.9	82	56.1	112	48.3
3	9	20.	10	13.7	21	4.4	30	13.7	11	9.	14	9.5	45	19.8
4	5	11.1	10	13.7	22	4.6	19	8.7	16	13.	8	5.4	21	9.
Total.....	45	100—	72	100—	472	100—	218	100—	123	100—	146	100—	232	100—

	44 cm.		46 cm.		48 cm.		50 em.		52 cm.		54 cm.		56 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
1	6	11.5	10	21.3	3	3.0	10	14.7	5	2.8	1	1.6	7	2.9
2	29	55.7	24	51.	42	42.4	18	26.4	29	16.5	9	14.4	19	8.
3	11	21.1	7	14.9	22	22.2	16	23.5	12	6.8	15	24.5	27	11.4
4	6	11.5	6	12.4	32	32.3	24	35.3	129	73.7	37	59.6	182	77.4
Total.....	52	100—	47	100—	99	100—	68	100—	175	100—	62	100—	235	100—

	58 cm.		60 cm.		62 cm.		64 cm,		66 cm.		68 cm.		70 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
1	6	4.2	5	5.2	2	4.1	2	4.2	3	4.2	2	3.6	1	0.7
2	29	20.4	11	11.5	4	8.1	7	14.9	12	17.1	6	10.9	13	10.3
3	25	17.6	14	14.7	2	4.1	9	19.1	8	11.4	4	7.2	11	8.7
4	82	57.8	65	68.4	41	83.7	29	61.7	47	67.1	43	78.1	101	80.1
Total.....	142	100—	95	100—	49	100—	47	100—	70	100—	55	100—	126	100—

	72 cm.		74 cm.		76 cm.		78 cm.		80 cm.		82 cm.		84 cm	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
1	10	9.2	5	4.7	1	2.2	1	1.4	10	4.1	1	0.8	0	0.0
2	7	6.4	13	12.2	4	8.8	4	5.7	21	8.6	5	4.0	4	2.2
3	14	13.	10	9.4	5	11.1	5	7.1	13	5.2	12	9.8	21	11.8
4	77	71.2	78	73.6	35	77.7	60	85.7	199	81.8	104	85.2	152	85.8
Total.....	108	100—	106	100—	45	100—	70	100—	243	100—	122	100—	177	100—

	86 cm.		88 cm.		90 cm.		92 cm.		94 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
1	1	.6	6	.9	1	1.8	2	.6	0	0.
2	3	1.8	23	3.6	1	1.8	3	.9	2	2.4
3	4	2.5	37	5.8	3	5.4	5	1.6	5	6.1
4	152	95.	570	89.6	50	90.9	300	96.7	74	91.3
Total.....	160	100—	636	100—	55	100—	310	100—	81	100—

In Figure 5, the percentages that strike the opening are taken as ordinates and the distances from the opening as abscissas. This curve makes it plain that on the whole, in spite of considerable fluctuations as the culture is moved back, the proportion of sporanges striking the opening sinks slowly from 98.4% at 2 centimeters to 88.3% at 26 centimeters, then somewhat more rapidly to 84.1% at 40 centimeters, then very rapidly to 19.3%

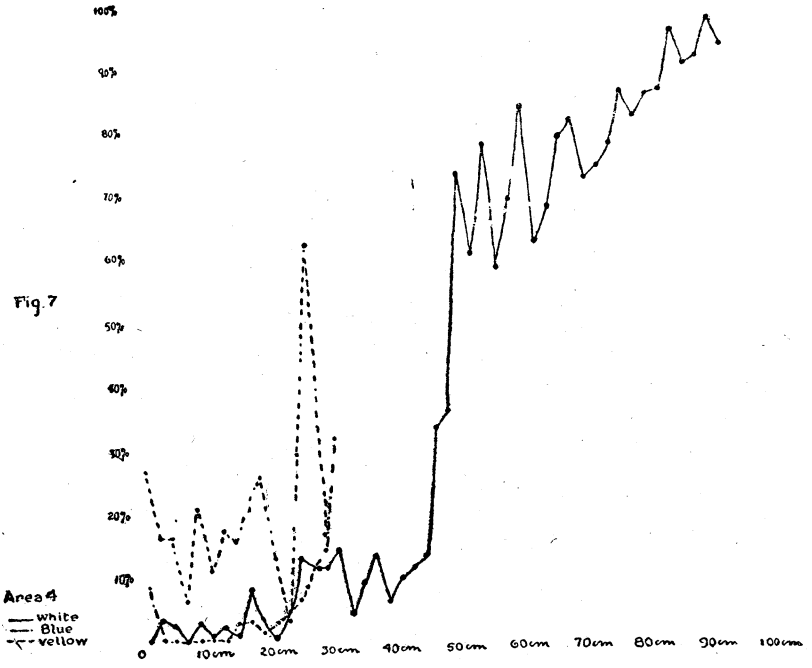

Fig. 7.

at 52 centimeters, and then very gradually again to 2.4% at 94 centimeters.

In Figure 6, the percentages of sporanges striking area 3 at the different distances were taken as ordinates. The percentages on this area increase irregular, 14 from 2% or 3% at short range to 23.5% at 50 centimeters, and then decrease irregularly to 6.1% at 94 centimeters.

In Figure 7, the percentage of sporanges striking area 4 were taken as ordinates. This curve rises very slowly from 0 at 2 centimeters to 5.4% at 40 centimeters, then abruptly to

73% at 52 centimeters, and then slowly again to 95% at 94 centimeters.

In general, it appears that as the distance of the culture from the source of light increases, there is a decrease in the proportion striking the outer areas. In interpreting these curves, the question arises whether at the greater distances the culture was entirely out of range. While the cultures vary in the initial velocity with which the sporanges are discharged, it has frequently been observed that they can reach an illuminated area at a distance of a meter vertically above them, and there can be little question that the diminishing percentage which reaches the opening in the series described is due directly to failure to allow for the effect of gravity on the path of the sporange.

To determine more exactly whether or not the sporanges fall below the opening due to the influence of gravity, the number of sporanges was recorded which were found above and below the median line already described, when the cultures were from 50 to 94 centimeters from the opening.

When the culture was at 50 centimeters distance, 58 sporanges were discharged. 16 sporanges (27.6%) were above the center of the opening and 42 (72.4%) below.

At 54 centimeters, 20% of the sporanges were above, and 80% below the middle.

At 82 centimeters only 11.4 were above the center.

As the culture was moved further from the opening, the percentage above the line irregularly decreased while that below the line increased.

The results of this series of observations are given in detail in Table II.

The large percentage below the median line with the culture from 50 to 94 centimeters distant corresponds to the decrease above noted in the number of sporanges which strike the opening at these distances. It is clear that more and more sporanges fall below the median line as the distance from the culture to the source of light increases.

The curves (Figs. 5, 6, and 7) showing the distribution of the sporanges zigzag back and forth through a rather wide range. This is at least partly due to the fact that it is impos-

sible to grow cultures which have the sporangiophores uniformly distributed over the surface. Preference was given to evenly distributed cultures when such were available, but many were used in which the majority of the sporaniophores were aggregated in the middle, at one side or around the edge. That the arrangement of the sporangiophores on the surface of the culture affects the records may be seen from the following experiments. In the apparatus described above, a culture was placed 110 centimeters from the opening, the center of the culture being exactly opposite the middle of the four centimeter opening. White daylight was used as before. The glass for catching the sporanges, instead of being against the opening, was placed at a distance of only ten centimeters from the culture. The culture chosen in this case had the majority of the sporanges arranged about the periphery in the shape of a horse-shoe, and it was placed so that the open side of the horse-shoe was downward. Figure 8 represents the results obtained. The configuration of the culture was reproduced with fair accuracy on the glass. It is manifest that if the horse-shoe had been placed with the other side up, the percentages in the different areas would have been markedly different. If the majority of the sporangiophores had been centrally instead of peripherally located, the percentage of sporanges striking the opening would have been greater.

On the following night the same culture was placed at a distance of ten centimeters from the opening. The majority of the sporangiophores appeared to be arranged essentially as on the preceding night. The glass was against the opening, that is ten centimeters from the culture as before. Figure 9 shows the result. There is a pronounced difference in the distribution of the sporanges in the two cases. In the former the configuration of the culture is evident; in the latter the arrangement of the sporanges does not indicate their position on the culture.

Comparison of these two results is interesting from another point of view. In the earlier experiments when the glass was kept at the opening, it was certainly true that a decreasing proportion of sporanges reached the opening as the culture was

TABLE II.—*Culture at 50—94 cm.—White light. Number of sporanges above and below the horizontal line A B drawn through the center of the opening.*

	50 cm.		52 cm.		54 cm.		56 cm.		58 cm.		60 cm.		62 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
Above	16	27.6	13	7.4	13	20	26	15.3	23	16	2	2.0	2	4
Below	42	72.4	162	92.6	52	80	209	84.7	120	84	93	97.9	47	96
Difference	—26	—44.8	—149	—87.2	—39	—60	—173	—69.4	—97	—68	—91	—95.8	—45	—92
	64 cm		66 cm.		68 cm.		79 cm.		72 cm.		74 cm.		76 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
Above	3	6.4	13	18.5	17	30.9	15	11.8	27	25	10	9.4	6	13.3
Below	44	93.6	57	81.5	38	59.1	111	88.1	81	75	96	90.6	39	86.7
Difference	—41	—87.2	—44	—63.0	—21	—38.2	—96	—76.2	—54	—50	—86	—81.2	—33	—73.4
	78 cm.		80 cm.		82 cm.		84 cm.		86 cm.		88 cm.		90 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
Above	3	4.2	33	13.1	14	11.4	32	18.6	46	28.7	19	2.9	5	9.
Below	67	95.3	210	86.9	108	88.6	145	81.4	114	71.3	617	97.1	50	91.
Difference	—64	—91.6	—177	—72.8	—94	—77.2	—113	—62.8	—68	—42.6	—598	—94.2	—45	—82.
	92 cm.		94 cm.											
	No.	Per ct.	No.	Per ct.										
Above	3	.9	7	8.6										
Below	307	99.1	74	91.4										
Difference	—304	—98.2	—67	—82.8										

moved further away, but this of itself does not prove conclusively a greater inaccuracy in aim; since a deviation of aim of a few degrees from the direction of the incident light might not prevent a sporange from hitting the opening at short range, but would prevent it if the distance from the opening were in-

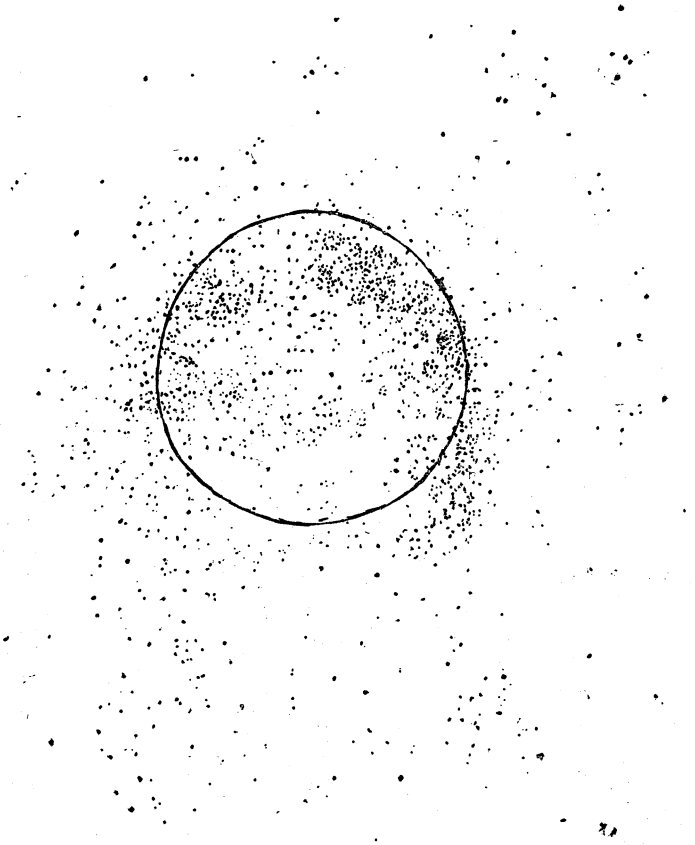

Fig. 8.

creased several fold. Here, however, we have positive evidence of greater inaccuracy of aim at long range. In each of these cases the sporanges have travelled 10 centimeters toward an opening 4 centimeters in diameter. In one case that opening was 10 centimeters away (Fig. 9), in the other 110 (Fig. 8). In the first case the sporanges are well within the opening and the scattering ones form an almost negligible percent-

age. In the second there is not only a large percentage outside the opening, but a considerable percentage outside of an area equaling in size the surface of the culture from which they started, showing that the accuracy of aim is appreciably diminished with the distance from the opening.

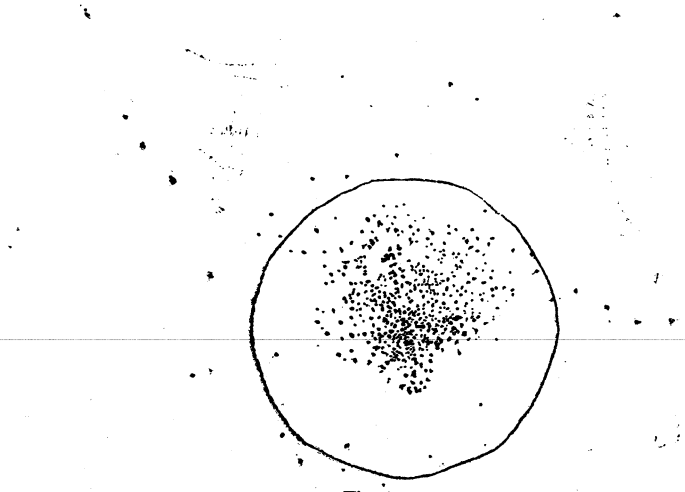

Fig. 9.

THE RESPONSE OF PILOBOLUS TO THE STIMULUS OF A SINGLE COLORED LIGHT.

Experiments were also made to test the accuracy of response of *Pilobolus* to colored lights as compared with white light. In these preliminary experiments, the light was admitted through plates of colored glass, and no attempt was made to obtain monochromatic light. When examined with the spectroscope, the blue glass used gave a mixed spectrum comprising all the violet, blue and green, together with a narrow band in the red. The yellow glass used transmitted only the red half of the spectrum, including red, orange, yellow and half of the green. The red glass let through only the red light. The apparatus and methods used were the same as for the experiments already described, except that a small plate of colored glass was placed inside the box against the opening, care being taken to exclude all light except that which came through the colored glass.

We shall describe first the experiments with blue light. The culture was first placed twenty centimeters from the circular opening, which was one centimeter in diameter, and remained there twenty-four hours. In making the records the target shown in Figure 1 was used. 261 sporanges were found on the glass. 161 (61.6%) were over the opening, area 1; 76 (29.1% on area 2;15 (5.7%) on area 3;5 (1.9%) on area 4; and 4 (1.5%) on area 5. In this case a larger percentage are on area 1 and a smaller percentage on area 2 than when white light was used.

In the next experiment the culture was placed twenty centimeters from an opening four centimeters in diameter, and the target shown in Figure 3 was used. The total number of sporanges on the glass at the end of twenty-four hours was 668. 524 (78.4%) were on area 1; 126 (18.8%) on area 2; 7 (1%) on area 3; and 11 (1.6%) on area 4. 97% were on the space over the opening, compared with 94.5% when white light was used under similar conditions. Only 18 of the 668 sporanges struck outside the opening.

Experiments with blue light were made with the culture at different distances from the four-centimeter opening. The same target was used as in the preceding experiment. At two centimeters distance 52.1% of the sporanges were on area 1 and 21.7% on area 2, making a total of 73.8% on the opening. Of the remaining 26%, 17% were on area 3. With the culture placed successively at distances from 4 to 14 centimeters from the opening, all the sporanges in each case were found on the opening. At 16 centimeters distance, 51% were outside the opening. As the distance from the opening increased still further, the proportion striking on the opening gradually decreased, until at 30 centimeters 76.8% were on the opening and 23.1% outside—for the most part below.

The results of these experiments are given in Table III. From the percentages on the different areas at the different distances, curves were plotted as in the previous experiments. Figure 5 (dot—dash line) shows the curve for the percentages on the opening. With the culture from 2 to 30 centimeters dis-

TABLE III.—*Culture at 2–30 cm. Opening 4 cm. in diameter. Blue light.*

	2 cm.		4 cm.		6 cm.		8 cm.		10 cm.		12 cm.		14 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
1.	12	52.1	17	68.	21	70.	19	57.6	20	80.	17	100.	31	88.5
2.	5	21.7	8	32.	9	30.	14	42.4	5	20.	0	0.	4	11.5
3.	4	17.3	0	0.	0	0.	0	0.	0	0.	0	0.	0	0.
4.	2	8.6	0	0.	0	0.	0	0.	0	0.	0	0.	0	0.
Total.....	23	100.—	25	100.	30	100.	33	100.	25	100.	17	100.	35	100.
	16 cm.		18 cm.		20 cm.		22 cm.		24 cm.		26 cm.		28 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
1.	365	63.4	127	59.	514	77.9	532	81.8	31	60.7	289	52.8	221	52.3
2.	189	32.9	53	24.6	126	19.1	77	11.8	16	31.3	118	21.5	127	30.
3.	17	2.9	9	4.1	7	1.0	26	4.0	2	3.9	69	12.6	30	7.1
4.	13	2.2	6	2.7	11	1.6	15	2.3	2	3.9	71	12.9	44	10.4
Total.....	574	100.	215	100.—	658	100.—	650	100.—	51	100.—	547	100.—	422	100.—
	30 cm.													
	No.	Per ct.												
1.	29	42.												
2.	24	34.8												
3.	7	10.1												
4.	9	13.0												
Total.....	69	100.—												

tant from the source of light, the curve closely follows that for the white light at the same distances. There is a gradual decrease in the percentage of sporanges on the opening as the distance from the culture to the opening increases. The curves for the percentages on areas 3 and 4 (Figs. 6 and 7, dot—dash line) also present a remarkable similarity to the corresponding curves for the same areas when white light was used. With the culture at 16 centimeters and at greater distances, the percentages on areas 3 and 4 gradually increase because the influence of gravity tends to make the sporanges fall below the opening. From these experiments it appears that *Pilobolus* fires its sporanges with about the same accuracy toward blue light as toward white light.

A yellow glass was next substituted for the blue, the culture being placed 20 centimeters from the opening one centimeter in diameter. The target shown in Figure 1 was used in counting the sporanges. The total number of sporanges found on the glass plate at the end of twenty-four hours was 52, of which 8 (15.4%) were on the opening, 20 (38.4%) were on area 2, 15 (30%) on area 3, 4 (7.8%) on area 4, and 5 (9.6%) on area 5. The percentages in the outer areas are rather large compared to those for the same areas, when the blue or white light is used. It appears at once that the reaction of *Pilobolus* to yellow light is much less accurate than to either blue or white light.

A four-centimeter opening was then tried in place of the one-centimeter opening. The target used in this experiment is the same as that shown in Figure 3. 98 sporanges were found on the glass, of which 32 (32.6%) were on area 1, 27 (27.3%) on area 2, 14 (14.2%) on area 3, and 25 (25.5%) on area 4. Thus 60% were on the opening, the remainder being widely scattered. In the corresponding experiments with blue and white light, 97.2% and 94.5% respectively were found on the opening.

The response to yellow light was further tested in a series of experiments with the opening four centimeters in diameter

and the culture from 2 to 30 centimeters distant. Data for this series of experiments are given in Table IV.

With the culture two centimeters from the opening, the total number of sporanges on the glass was 38. 15.8% were on area 1 and 39.4% on area 2, making 55.2% on the opening. 18.4%were found on area 3, and 26.3% on area 4.

At 10 centimeters from the opening, of the 198 sporanges fired in twenty-four hours, 51% were on area 1, 16.6% on area 2, 7.5% on area 3, and 24.7% on area 4. In this case 67.6% were on the opening.

At 20 centimeters distance, 98 sporanges were found on the glass. 32.6% reached area 1; 27.3% were on area 2. At this distance, then, 59.9% were on the opening, 14.2% were on area 3, and 25.5% on area 4.

With the culture 30 centimeters distant, only 30 sporanges were fired. 30% of these were found on area 1 and 23.3% on area 2, making 53.3% on the opening. 30% were on area 3, and 16.6% on area 4.

The curve plotted from the percentages of the sporanges on the opening (Fig. 5 dash—dash line) and on areas 3 and 4 Figs. 6 and 7, dash—dash lines) vary markedly from the corresponding curves for the blue and white lights. The largest percentage found on the opening was 88.3% at eight centimeters distance. From this point there was a decrease down to 53% at 30 centimeters. At no point was the percentage on the opening in the yellow light equal to that on the same area in the blue and white lights.

In making these countings in the yellow light, the numbers of sporanges above and below the horizontal median line (A B, Fig. 3) were recorded. The majority of the sporanges are always found below the line A B. With the culture 12 centimeters from the opening the number of sporanges below the line A B exceeds that above by 2%, at 24 centimeters by 10%, and at 30 centimeters by 26.7%. The number of sporanges above and below the line at the various distances are recorded fully in Table V.

TABLE IV.—*Culture at 2–30 cm. Opening 4 cm. in diameter. Yellow light. Target Figure 3.*

	2 cm.		4 cm.		6 cm.		8 cm.		10 cm.		12 cm.		14 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
1.	6	15.8	4	12.5	71	41.2	60	57.6	101	51.	28	58.3	17	48.6
2.	15	39.4	19	59.3	60	34.8	32	30.7	33	16.6	12	25.	11	31.4
3.	7	18.4	4	12.4	14	8.1	4	3.8	15	7.5	3	6.4	1	2.8
4.	10	26.3	5	15.6	27	15.7	8	7.6	49	24.7	5	10.4	6	17.1
Total.....	38	100.—	32	100.—	172	100.—	104	100.—	198	100.—	48	100 —	35	100.—

	16 cm.		18 cm.		20 cm.		22 cm.		24 cm.		26 cm.		28 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
1.	72	54.5	80	36.5	32	32.6	23	40.3	20	52.7	13	25.	23	6.7
2.	43	32.5	59	26.9	27	27.3	17	29.8	7	18.4	11	21.1	42	12.3
3.	15	11.3	32	14.5	14	14.2	10	17.5	10	26.3	13	25.	65	19.
4.	2	1.5	58	26.4	25	25.5	7	12.2	1	2.6	15	28.8	211	61.8
Total.....	132	100.—	219	100.—	98	100.—	57	100.—	38	100.—	52	100.—	341	100.—

	30 cm.	
	No.	Per ct.
1.	9	30.
2.	7	23.3
3.	9	30.
4.	5	16.6
Total.....	30	100.—

Culture at 2–30 centimeters. Yellow light. Number of sporanges above and below A B. Target, Figure 3.

	2 cm.		4 cm.		6 cm.		8 cm.		10 cm.		12 cm.		14 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
Above	3	7.9	13	40.6	74	43	44	41.9	93	49.4	24	48	17	48.5
Below	35	92.0	19	59.3	98	56	61	58	95	—50.5	25	50	18	51.4
Difference	—32	—84.0	—6	18.7	—24	—13	—17	—16.1	—2	—1.1	—1	—2	—1	—2.9

	16 cm.		18 cm.		20 cm.		22 cm.		24 cm.		26 cm.		28 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
Above	62	46.9	102	46.5	43	43.8	20	35	17	44.7	21	40.3	135	39.5
Below	70	53	117	53.4	55	56.1	37	64.9	21	55.3	31	59.7	206	—60.4
Difference	—8	—6.1	—15	—6.9	—12	—12.3	—17	—29.9	—4	—10.6	—10	—18.4	—71	—20.9

	30 cm.	
	No.	Per ct.
Above	11	36.6
Below	19	63.3
Difference	—8	—26.7

The accuracy of response to red light was tested in one experiment. The culture was placed 20 centimeters from an opening one centimeter in diameter. A red glass was placed inside the box against the opening. The target shown in Figure 1 was used to count the sporanges. The number of sporanges on the glass after twenty-four hours was 80. 1.2% of these were on area 1, corresponding to the opening; 13.7% were on area 2; 28.7% on area 3, 18.7% on area 4, and 37.5% on area 5. The reactions toward red light are evidently very vague and uncertain.

The results which have now been described indicate that *Pilobolus* aims point blank at a source of light, making no allowance for the distance through which the sporanges must travel; that is, it does not aim higher for a distant source of light than for a near one. To test this point still further, the culture was placed in the same box with its center on a level with the middle of a four-centimeter opening. As in a previous experiment, it was placed on successive days at different distances along the median line of the box. The glass upon which the sporanges were received was, however, always placed at a distance of 25 centimeters from the culture; thus if the sporangiophores aim higher as the distance from the source of light increases, the sporanges should strike the glass above the target when the culture is sufficiently far from the source of light.

The target used in counting the sporanges is shown in Figure 10. In this case the field was divided off into horizontal strips one centimeter wide, and they were numbered upward and downward from a line running through the center of the opening, the strip above being marked + and that below—. An area 14 centimeters wide was found to include nearly all the sporanges; those beyond this area were counted under the head "7+"

The culture was placed successively at 25, 35, 40, 50, 65, 80, 90, 100 and 110 centimeters from the opening, being left twenty-four hours in each position. With the culture at 25 centimeters from the opening, the center of distribution of the

sporanges is 0.6 centimeter below the median line. At 35 centimeters it is 0.3 centimeter below; at 40 centimeters, 0.4 centimeter below; at 50 centimeters, 0.5 centimeter below; at 65 centimeters, 1.3 centimeters below; at 80 centimeters, 1.2 centimeters below; at 90 centimeters 1.1 centimeters below; at 100 centimeters, 0.7 centimeter below; and at 110 centimeters, 0.9 centimeter below. The data are given in complete form in Table VI. The center of distribution varies from 0.3 centimeter to 1.3 centimeters below the median line; in no case is it above. It thus appears that the sporangiophores are pointed directly at a source of light, whatever the distance of the latter.

TABLE VI.—*Glass 25cm. from Culture.*

Culture at	25 cm.	35 cm.	40 cm.	50 cm.	65 cm.	80 cm.	90 cm.	100 cm.	110 cm.
+7†	0	2	1	0	0	0	0	1	3
+7	0	0	2	0	0	0	0	2	5
+6	0	0	1	0	0	0	0	1	4
+5	1	1	3	0	0	1	0	3	0
+4	1	1	1	0	0	1	0	4	9
+3	2	3	3	0	0	13	0	2	9
+2	15	63	16	7	2	17	0	43	32
+1	167	204	47	32	15	49	13	134	70
−1	601	345	125	61	21	145	25	138	178
−2	64	105	70	48	54	223	22	149	303
−3	13	10	12	15	23	110	22	119	78
−4	6	6	1	0	3	31	0	12	4
−5	2	2	4	0	1	16	0	2	1
−6	2	1	0	0	1	3	0	3	3
−7	1	3	0	0	0	5	0	0	3
−7+	0	0	0	0	0	6	0	1	1
Total	856	746	285	163	120	622	82	614	693
Position of center of firing	−.6	−.3	−.4	−.5	−1.3	−1.2	−1.1	−.7	−.9

A further series of experiments was made to determine graphically the path of the sporanges as they are discharged toward the light. From the experiments already described it is clear that as the distance from the source of light increased, the sporanges reached the glass at a lower point. But in these cases the intensity of the stimulus diminished with the increased distance. In the present series the culture was kept at 110 centimeters from the opening and the sporanges were intercepted by a glass at various points in their path on the way to the opening. The intensity of the light thus did not vary with the distance of the culture from the glass. Twenty-four hour

exposures were made, with the glass successively at 10, 20, 35, 50, 65 and 85 centimeters from the culture. The target described in the preceding experiment (Fig. 10) was used in recording the distribution of the sporanges. When the glass was ten centimeters from the culture, the center of distribution of the sporanges was 0.9 centimeter below the median line. With

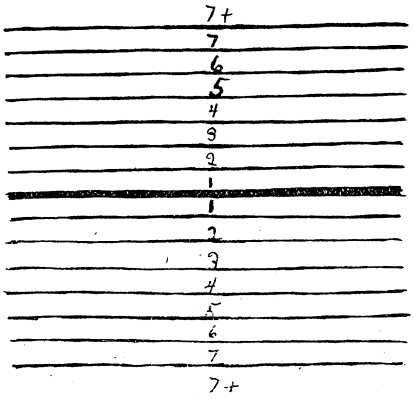

Fig. 10.

the glass 25 centimeters from the culture, the center of distribution was 1.1 centimeters below; and with the glass at 35 and at 50 centimeters, the center of distribution was in each case 1.2 centimeters below. The center of distribution sinks to 6.2 centimeters below the median line with the glass at 65 centi-

TABLE VII.—*Culture 110 cm.*

Culture at.....	10 cm.	25 cm.	35 cm.	50 cm.	65 cm.	85 cm.
+7+.....	0	0	2	0	1	2
+7.....	0	0	0	1	0	0
+6.....	0	0	0	0	2	1
+5.....	0	3	2	0	4	1
+4.....	0	3	1	2	8	2
+3.....	0	9	1	2	64	5
+2.....	0	14	8	20	194	27
+1.....	26	52	30	27	205	43
−1.....	49	178	87	68	281	46
−2.....	48	222	129	90	173	29
−3.....	27	98	116	79	116	44
−4.....	4	24	59	84	107	31
−5.....	0	3	32	30	87	32
−6.....	0	2	12	15	65	22
−7.....	0	1	10	11	63	26
−7+.....	0	6	12	11	91+521	61+344
Total.....	154	615	501	438	1,982	816
Center of distribution of sporanges.....	−9	−1.1	−1.2	−1.2	−6.2	−124

meters, and to 12.4 centimeters below with the glass at 85 centimeters. The results are given in detail in Table VII. Under "7+" is given the number of sporanges that did not reach the vertical glass plate, but fell on a glass on the floor of the box.

When plotted (Fig. 11), these data do not give the modified parabolic curve of the path of a projectile, for the descent once begun is not rapid enough. The path of the individual sporange however is probably that of a projectile.

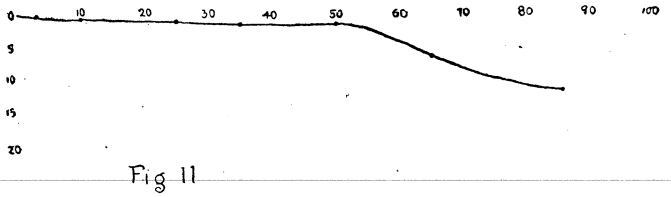

Fig 11

Fig. 11.

The Response of Pilobolus When Subjected to Two Simultaneous White Lights of Equal Intensity.

A preliminary experiment was made to determine the general nature of the response of *Pilobolus* to two simultaneous light stimuli, using a box with two openings in one end. The box was of cardboard, light-proof, painted black inside, and was 42 centimeters long, 14 centimeters wide and 9 centimeters high. In one end were two circular holes 7.5 centimeters apart, each being 1.25 centimeters in diameter and 5.5 centimeters above the bottom. The glass was placed inside the box, against the end in which the openings were cut.

The culture of *Pilobolus*, in a two-inch flower-pot as before, was placed on its side with its surface vertical and facing the end of the box containing the openings. The center of the surface was on the level of the centers of the openings and was equally distant (32 centimeters) from each. The experiment was set up at night. Twenty-four hours later, a drawing was made, showing the distribution of the sporanges on the glass before the openings (Fig. 12). For purposes of comparison, a control experiment was set up exactly like the first except

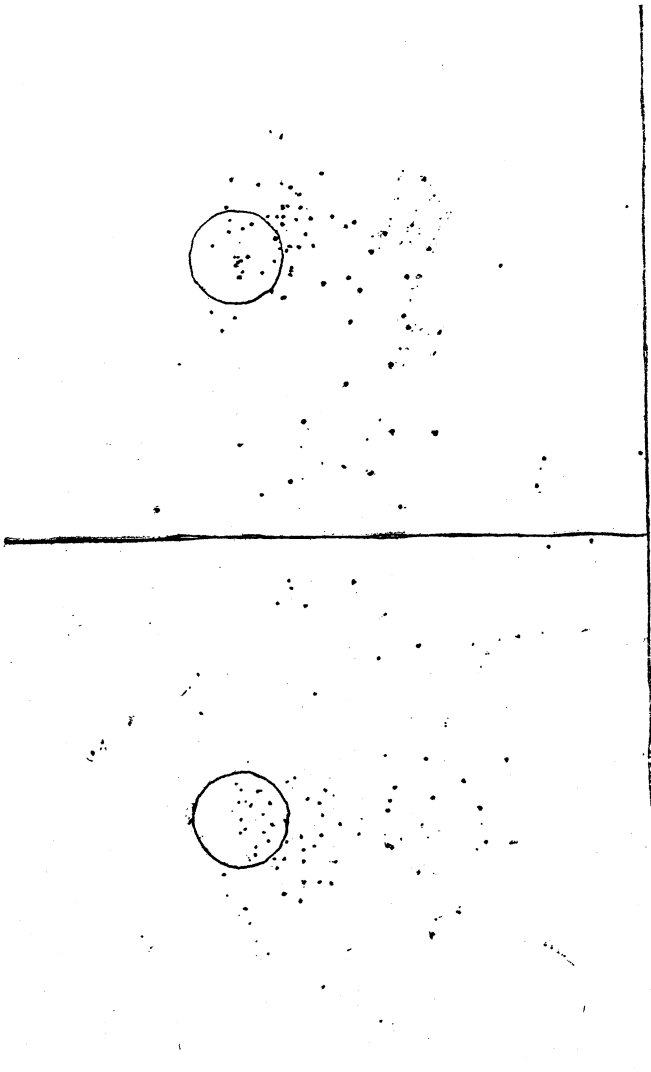

Fig. 12.

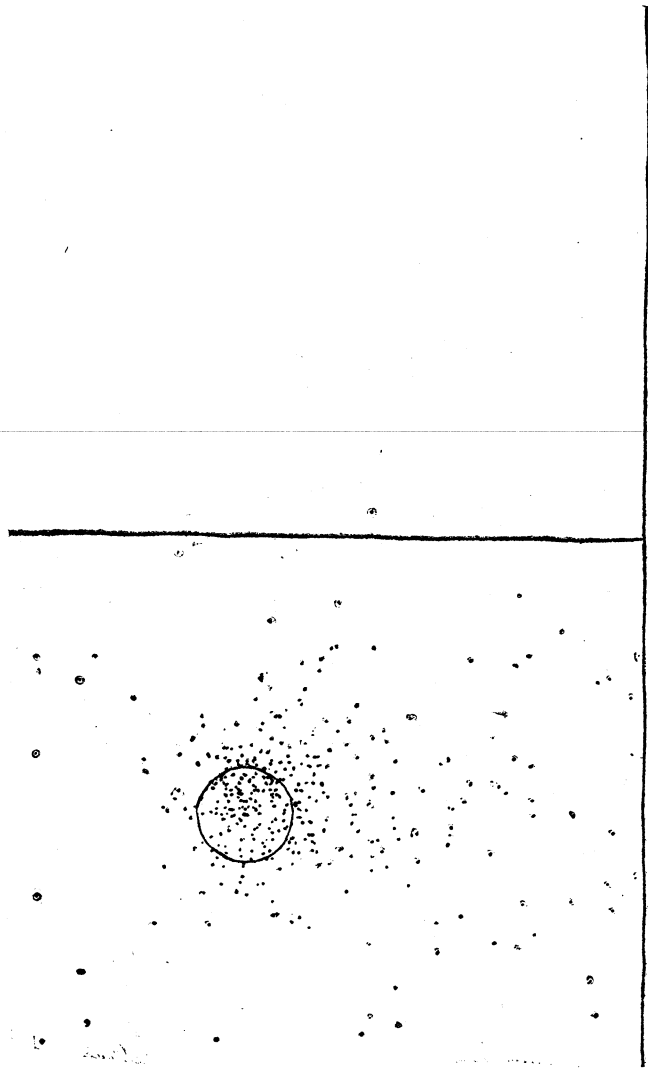

Fig. 13.

that the right-hand opening was closed by a black pad. Figure 13 shows the result.

If, when subjected to two simultaneous beams of light, a sporangiophore reacts to but one, and if the reaction to that stimulus is in no way influenced by the presence of the other, then Figure 13 should be comparable in every detail to the left half of Figure 12. The absolute number of sporanges in a given area would vary of course with the total number discharged in the course of the experiment, but the percentages in the corresponding areas should be substantially equal.

For convenience in comparing the results of the two experiments, the field is first divided into right and left halves by a vertical line midway between the two openings. Each half is then subdivided into three areas by vertical lines tangent to the right and left edges of the openings. The sporanges in each area were counted and the percentage was calculated of the total number included in each area. To make Figure 13 directly comparable to the left half of Figure 12, the total percentage for Figure 13 was made 50 instead of 100.

TABLE VIII—*Tabulation of results shown in figures 12 and 13.*

	Left half of field.			Right half of field.			Total
	To left of opening	On opening or below	To right of opening	To left of opening	On opening or below	To right of opening	
Sporanges.....	8	40	17	19	46	13	143
%.....	5.6	28	11.9	13.3	32.1	9.1	
Sporanges.....	42	204	82	.....	.....	.....	328
	6.4	31.1	12.5		.....	.....	

As the table shows, there is a very close correspondence between the two experiments as to the proportional number of sporanges in each area.

There is, however, one difference between the results of the two experiments which the table does not show. When there is but one source of light, (Fig. 13), the sporanges which strike the target at the lowest level fall, of course, approximately below the opening. When there are two sources of light, the

lowest sporanges strike in the neighborhood of the vertical line midway between the two openings. In Figure 12, the sporanges as a whole are arranged roughly in the form of a crescent with the convex side below and the points of the crescent upon the openings. This crescent shape has been repeatedly noted in other experiments of a similar nature. The sporanges which are aimed directly at one or the other of the two openings fall very little below. Why the few sporanges which drop far below the level of the openings should be the ones which aim between the two openings is unexplained. It may be that the weaker, inferior individuals are less sensitive to light than the rest, and that they perceive in the two lights but one general source of illumination, and aim accordingly.

The majority of the sporanges aim at one source of light or the other and aim as accurately at the one chosen as though the other did not exist. In general, it appears that under the conditions of the experiment, a sporangiophore reacts to one only of two simultaneous lights.

In the next series of experiments, we studied the variations in the distribution of the sporanges as the distance between the two openings varied. A light-proof wooden box was constructed, provision being made in one end for two openings which could be shifted so as to be placed at any desired distance apart but always at the same level. The interior of the box was painted black. Daylight was used in these experiments, and the box was placed before a south window with the end containing the openings facing south. The position of the box was not changed during the series of experiments. Within the box, the culture, in a two-inch flower-pot, was supported on a black stand. The flower-pot lay on its side, with the surface of the culture vertical and facing the end of the box containing the openings. The center of the culture was on a level with the center of the openings. In the experiments, the distance between the openings (measured from the center of one opening to the center of the other) was varied between 2 and 26 centimeters, but the distance from the culture to each

opening was kept constantly at 42 centimeters. The drying out of the culture was to some extent prevented by supporting a dish of water on the stand just below the culture.

The sporanges were caught on a glass plate placed inside against the end of the box, and their position on and about the openings was recorded on a sheet of paper divided into equal areas by parallel vertical lines one centimeter apart. The openings are represented by two circles one centimeter in diameter. Figure 14 shows the chart for an experiment with the openings 10 centimeters apart. The number of centimeter-wide areas between the openings of course increased as the openings were moved farther apart. The number of sporanges striking within each area was recorded separately.

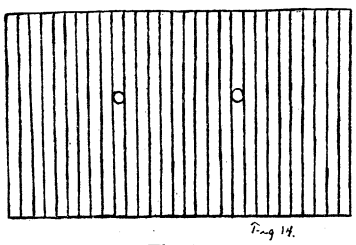

Fig. 14.

We found in these cases, as in the preliminary experiments, that a large proportion of the sporanges landed in the vicinity of one or the other of the two openings. Throughout the series (Table IX) in which the distance from the center of one opening to the center of the other varied from 2 to 26 centimeters, fully three-fourths of the total number of sporanges fell upon the vertical areas which included the openings or within 2 centimeters to right or left.

When the centers of the openings were only 2 centimeters apart, the two groups of sporanges were not distinct. They overlapped so as to present the appearance of a single broad-centered group. Of a total of 201 sporanges, 39 were on the area containing one opening, 49 on the other, and 52 sporanges on the area between the two. (Table IX).

In the first five experiments of the series (Table IX), in which the distance between the openings was increased from

two to seven centimeters, there were more sporanges on a strip midway between the openings than on either strip containing an opening. Even in these cases, however, the position of the openings could be located in the group of sporanges on the glass by the presence of two more densely crowded areas. The sporanges between the openings were more scattered. Many lay far below the level of those on the strips which included the openings. The crescent shape of the whole group, which appeared in the preliminary experiment, first became apparent when the openings were five or six centimeters apart. The broad middle part of the crescent resulted of course from the fact that in that region (as noted in the preliminary experiments) the sporanges were scattered through a greater vertical distance than were those in the immediate neighborhood of the openings.

As the openings were placed seven centimeters and more apart, however, a marked decrease appeared in the proportion of sporanges found in the area between the openings. The single crescent-shaped group became gradually resolved into two groups separated from each other by a broad region containing relatively few sporanges. From 70 to 80% of the total number were found on the strips containing the openings or within two centimeters to right or left, and the remainder were scattered over the areas between the openings. There were fewer in the middle areas, but there was no central vacant space.

Not until the openings were 17 centimeters apart did a central space appear comparatively free from sporanges. At this stage in the experiments, the included angle between the two sets of light rays reaching the culture was 23° or 24.° A noteworthy peculiarity lay in the fact that when the clear central space first appeared, it was not, as might be expected, only a centimeter or so wide, and thereafter a centimeter wider with each increase in the distance between the openings. On the contrary, the clear median space, when it first appeared, was about ten centimeters wide. When the centers of the openings were 15 centimeters apart, the sporanges in the vertical areas midway between the openings were, respectively,

7—7—2—3—4—5—3—4

Total, 35=10% of the total number (340) of sporanges in this experiment.

When the centers of the openings were 16 centimeters apart, the corresponding areas contained, respectively,

6—5—0—2—1—1—3—2—6

Total, 26=8% of the total number (315) of sporanges fired.

Compare with these, the numbers of sporanges found in the median region of 10 strips, when the distance between the centers of the openings was 17 centimeters:

0—0—1—1—0—0—1—1—1—0

Total, 5=3% of the total number (151) of sporanges fired.

In the remaining experiments, as the distance between the openings was increased still further, the two discrete groups were maintained and the intervening area grew correspondingly wider.

The complete data of this series of experiments are given in Table IX. Each horizontal row of figures in this table represents the whole number of sporanges striking the glass in a day, that is in one experiment. The total for each experiment is given at the left. The numbers in bold-faced type *in each* row represent the strips which include the openings. Between the records for these two areas in each case are given in order the number of sporanges found in each of the centimeter-wide strips between the two openings. Reading outward from the area covering each opening, the figures represent the contents of 9 similar areas, the first 4 being recorded separately, and the five outermost (comprising usually a very small number of sporanges) being given collectively. The angle between the two sets of light rays reaching the culture has been computed in some cases and is given at the left of the table in the column immediately following the totals.

Table XI.

Distance between centers of openings.	Total.	Angle beween two sets of light rays.																																						
2	201	2¾°													3	5	3	7	14	39	52	49	16	9	2	1	1												
3	71														2	1	1	3	3	10	16	16	8	3	1	3	0	4											
4	102													2	0	0	0	3	20	14	24	14	12	8	1	0	1	3											
5	87	6¾°												2	1	0	2	8	15	14	14	11	9	3	0	3	1	2	2										
6	120												0	1	1	0	7	13	17	18	32	18	6	4	2	1	0	0	0										
7	438												5	1	4	20	36	57	58	39	32	30	31	32	39	34	7	2	1	8									
8	220											2	1	2	6	8	24	14	21	20	13	22	28	22	16	4	7	2	3	6									
9	295											0	1	5	19	41	36	29	17	6	8	11	10	19	36	22	25	6	2	0	3								
10	181	13¼°									2	0	2	11	17	37	30	18	10	7	7	1	5	2	13	5	11	0	2	0	1								
11	151										1	2	0	4	13	27	23	16	12	5	0	4	6	3	7	6	9	9	2	1	0	1							
12	71									0	0	1	0	6	10	20	8	4	1	4	4	2	2	2	1	1	3	1	1	0	0	0							
13	140									1	1	3	3	9	23	26	13	4	3	9	3	7	9	0	2	4	10	5	4	1	0	0	0						
14	331								1	2	0	14	34	74	59	28	12	6	6	5	3	3	4	6	12	19	21	11	4	4	2	0	1						
15	340	20°							2	6	8	17	53	70	40	40	14	7	7	2	3	4	5	3	4	9	16	11	12	2	1	1	2	1					
16	315							2	3	7	12	29	46	40	19	8	6	5	0	2	1	1	3	2	6	21	27	38	19	15	3	0	0	0					
17	151							1	0	0	6	18	14	16	10	5	0	0	1	1	0	0	1	1	1	0	13	13	19	14	13	2	1	0	1				
18	113						1	0	0	3	4	7	13	12	2	1	0	0	1	3	0	1	0	0	1	2	4	14	21	16	3	3	1	0	0				
19	62						0	0	0	1	9	12	10	1	1	0	0	1	1	0	2	1	3	0	0	0	0	3	6	4	5	2	0	0	0	0			
20	94	27½°				1	0	0	1	9	18	18	9	0	0	2	0	0	0	0	0	0	1	0	0	0	0	3	9	11	6	5	1	0	0	0			
21	39					0	0	0	0	0	6	9	4	1	0	0	0	1	0	1	2	1	1	0	0	0	0	0	2	2	4	2	1	1	1	0	0		
22	50				0	0	0	1	4	7	10	5	1	1	0	0	0	0	1	0	0	0	1	0	0	0	1	1	1	2	8	3	4	0	0	0	0		
23	51				0	0	0	1	4	4	3	4	1	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	2	15	10	4	0	0	0	0	1	
24	588			3	0	0	6	43	74	66	48	23	6	1	0	0	0	1	0	0	0	0	0	0	1	1	1	2	2	51	80	121	96	58	4	1	1	1	
25	447	36°		4	1	1	24	48	92	75	55	9	7	1	1	0	0	0	1	0	0	0	1	0	1	0	0	0	1	9	28	37	28	48	22	0	1	0	0
26	204		1	1	0	2	10	23	24	18	13	2	0	0	0	1	0	1	1	0	0	0	0	0	0	1	0	1	2	1	11	17	26	28	11	2	4	2	1

The Response of Pilobolus to the Stimuli of two Successive White Lights of Equal Intensity.

The object of our next series of experiments was to determine to what extent the sporangiophores can change their aim during the morning, and whether the discharge of the sporanges can take place while a heliotropic reaction is in progress.

The sporangiophores in a culture are not all at the same stage of development at the same time. This is well seen in the evening when the tip of the sporangiophore is swelling to form the sporange. Upon some sporangiophores, the only indication of a sporange at this time is a small yellow knob, while on others immediately adjacent the sporanges are full-sized and turning dark. The latter are something more than an hour ahead of the former. In consequence of these differences, the sporanges do not mature at the same time and are not discharged at the same time. It requires at least an hour for the discharge of the entire crop. At any particular moment during the time of discharge, some are on the point of discharging, others will discharge in a few minutes, and still others may not discharge for an hour.

The cardboard box used in the preliminary experiments upon simultaneous stimulation was used for these experiments. The two holes in the end of the box were 1.25 centimeters in diameter, 7.5 centimeters apart, and 5.5 centimeters from the bottom. In order that the results might be as clean-cut as possible, the distance from the culture to the openings was made comparatively small—15 centimeters. The culture was laid on its side on the bottom of the box, with the surface of the culture facing the openings and equidistant from them. The culture was propped securely at the sides to prevent its rolling over should the box happen to be jarred. As before, the sporanges were caught on a glass plate placed inside against the end of the box. The form of chart from which the records were made is given in Figure 15. A vertical line down the center of the field divides it into right and left halves and each half is subdivided by a vertical line through

the center of the opening. The sporanges falling upon each opening are recorded separately. As the distance from the culture to the openings is short, very few sporanges fall below the openings.

At night, when the experiment was set up, the opening, in the right half of the field was carefully closed by a black pad and left so over night. The morning light entered the box through the left opening only. At a definite time in the morning, the glass was removed from the box, and a record made of the sporanges already received upon it; the black pad was then shifted to the left opening, thus uncovering the right opening, and the glass was cleaned and replaced. Care was taken not to jar the culture, and the box was kept closed while the record was being made. In the evening of the same day a second record was made.

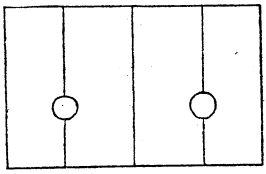

Fig. 15. Chart used in recording response of Pilobolur to two successive white lights.

By conducting the experiments as just described, the mature or nearly mature sporangiophores which have been exposed to light from one source are subjected suddenly to light from another source; that from the former source being cut off. The distribution on the glass of the sporanges at the end of the experiment will measure the ability of sporangiophores at this stage of development to perceive and react to light stimuli.

This work was done during the short days of November (16 to 27); at this time of year there was little or no discharge of sporanges before 8:00 A. M. The exact time of the beginning of the discharge varied greatly with different cultures; thus, one had discharged nearly half the total number of sporanges at 8:30 A. M., while another had not begun to discharge at 10:15 A. M. These variations may have been

due to differences in the morning illumination, to weather conditions, or to the fact that some cultures were shaded more than others on the afternoon before being used. That the light supply had something to do with it seems probable, because the development of a crop of sporanges can be retarded considerably by placing the culture in a dark room during the afternoon and night.

In the experiments in which the light was changed at 7:15, 7:30, 8:00, 9:45 and 10:15, no sporanges had been discharged at the time of the change. (Table X). In the other experiments of this series, some of the sporanges were found upon the glass in the morning. In the latter cases, the results were of the same character as those previously obtained when a single light was placed a little to one side of the culture (Fig. 13). The culture was closer to the opening in the present series, and so the sporanges were better centered about the opening. Only about one-eighth of the sporanges (13%) lay between the opening and the middle line of the field. Very few sporanges lay in the right half of the field.

Of the sporangiophores still carrying sporanges when the position of the light was changed, the majority began to bend over toward the new source of light and in many cases the reaiming was completed before their discharge.

The experiment (Table X) in which the direction of the light was changed at 9:15 A. M. may be taken as a typical example. When the box was opened at 9:15 A. M., we found 63 sporanges on the glass. Of these, 40 were upon the opening, 8 to the left, and 10 to the right but still in the left half of the field, while 5 scattered sporanges were on the right half of the field. The glass was cleaned and replaced, the left-hand opening closed and the right-hand one opened.

In the evening, we found the remainder of the crop of sporanges (68) on the glass. The distribution of these was as follows: (*1* is the closed left-hand aperture, *2* the open right-hand aperture):—

7	To left of *1*	Left half of field.
13	On *1*	
19	To right of *1*	
8	To left of *2*	Right half of field.
18	On *2*	
3	To right of *2*	
68	Total	

The percentages (Table XI) that these number form of the total (68) are as follows:

10.3	To left of *1*	Left half of field.
19.1	On *1*	
27.9	To right of *1*	
11.7	To left of *2*	Right half of field.
26.4	On *2*	
4.4	To right of *2*	
100.0	Total	

About 40% of the sporanges fired after the change in the source of illumination are in the right half of the field, clustered about the new source. This 40% come from sporangiophores which have completed a heliotropic reaction, although half of the crop of sporanges had been discharged at the time the new stimulus was presented. 28% are between the former opening and the middle of the field. This is a larger proportion than would have been found in this part of the field had there been no change in the direction of light. (Compare Table VIII, which shows 12% in this part of the field). It follows that the sporangiophores already aimed at *1* began to swing over toward the new source of light and that the sporanges were discharged in some cases before the new heliotropic reaction had proceeded to its completion.

A complete record of this series of experiments is given in Table X. From the records made in the evening (Part 2 of Table X) the percentages found in the different areas in each experiment have been calculated (Table XI). Each horizontal row of figures represents one experiment, and in the column at the left is given the fraction of the total crop of sporanges which was found in the evening record.

TABLE X. PART 1.—*Showing distribution of sporanges in the morning when right opening had been closed.*

Time when examined. A. M.	Left half of field.			Right half of field.			Total.
	Left of opening.	Opening.	Right of opening.	Left of opening.	Opening.	Right of opening.	
7:15	0	0	0	0	0	0	0
7:30	0	0	0	0	0	0	0
8:00	0	0	0	0	0	0	0
8:30	0	53	1	0	0	0	54
9:00	2	16	2	0	0	1	21
9:15	8	40	10	3	0	2	63
9:30	4	79	11	4	0	1	99
9:45	0	0	0	0	0	0	0
10:00	4	3	0	0	0	0	7
10:15	0	0	0	0	0	0	0
10:30	13	28	8	2	0	0	51

TABLE X. PART 2.—*Showing distribution of sporanges in the evening. At time indicated the left opening was closed and the right opened.*

Time of changing light. A. M.	Left half of field.			Right half of field.			Total.
	Left of opening.	Opening.	Right of opening.	Left of opening.	Opening.	Right of opening.	
7:15	1	0	3	58	86	13	161
7:30	0	1	2	15	38	12	68
8:00	7	3	10	12	37	13	82
8:30	2	1	21	5	17	1	47
9:00	5	13	19	6	13	0	56
9:15	7	13	19	8	18	3	68
9:30	15	3	5	8	14	0	45
9:45	0	0	1	33	109	18	161
10:00	2	3	5	10	12	4	36
10:15	4	0	1	31	11	3	50
10:30	9	15	31	13	28	6	102

In the experiment in which the light was shifted at 9:45, no sporanges had been discharged at the time the change was made. In the evening, one sporange only was found on the left half of the field, showing that all of the sporangiophores but one had reacted to the new stimulus. The re-aiming was nearly as perfect as the original aim, 67.7% being found upon the new opening, 11.2% beyond it to the right, and 20.5% between the opening and the middle line of the field.

In the experiment in which the light was changed at 10:15 A. M., the re-aiming was less perfect. In this case, also, no sporanges had been discharged at the time the light was changed, but some of the sporanges were later discharged while the sporangiophores bearing them were still directed at the

former source of light. We found 10% of the sporanges on the left half of the field. On the right half only 22% were on the opening and 6% beyond it, but 62% were found between the middle line and the new opening. The discharge of these sporanges evidently took place while the re-aiming was in progress.

TABLE XI.—*Percentages calculated from table X. part 2.*

	Fraction of total crop of sporanges in evening record.	Left half of field.			Right half of field.		
		Left of opening.	Opening.	Right of opening.	Left of opening.	Opening.	Right of opening.
9:45	1	0	0	.6	20.5	67.7	11.2
7:15	1	.6	0	1.9	36.0	47.2	1.2
10:15	1	8.0	0	2.0	62.0	22 0	6.0
7:30	1	0	1.5	3.	22.1	55.9	17.6
8:00	1	8.5	3.7	12.2	14.6	45.9	15.9
10:00	5/6	5.5	8.3	13.9	27.8	33.3	11.1
9:00	5/7	9.0	23.2	34.0	10.7	23.2	0
10:30	2/3	8.8	14.7	30.4	12.7	27.4	5.9
9:15	1/2	10.3	19.1	27.9	11.7	26.4	4.4
8:30	4/9	4.3	2.1	44.7	10.6	36.2	2.1
9:30	5/16	33.3	6.7	11.1	17.7	31.1	0

In six of the experiments, those namely in which the light was changed at 10:00, 9:00, 10:30, 9:15, 8:30, and 9:30, part of the sporanges were already discharged. Under these conditions, a considerable proportion of the remaining sporanges were about to be fired at the moment the change was made and others were to be fired in a few minutes. Consequently, a larger proportion of the sporanges fired after the change were discharged in the direction of the original illumination. Large numbers, however, are found in the region between the openings, and a varying proportion reach the new opening.

The 8:30 experiment is typical. Nearly half of the entire crop of sporanges was discharged when the light was changed. Of the remaining half, we found in the evening that 4% were to the left of the former opening, 2% on it, 44.7% between the left opening and the middle line of the field, 10% between the middle line and the new opening, 36% on the new opening, 2% beyond it to the right. Late as it was, very few

sporangiophores failed to begin to respond to the new stimulus, and nearly half of them completed the reaction.

These data demonstrate that perception and reaction can take place in fully mature sporangiophores; that a heliotropic bending can take place when the sporange is about to be discharged, and that the reaction can be interrupted at any point by the discharge.

We next attempted to determine to what extent sporangiophores, subjected to two light stimuli, turn toward the nearer source of light. The surface of the culture used was five centimeters in diameter. The culture being placed, as before, equidistant from the two openings, if the openings admit exactly the same amount of light, only those sporangiophores which happen to be on the median vertical line of the culture will be equally lighted from the two directions. Placed at either side of the middle line, the sporangiophore will be nearer to one opening and farther from the other. The following experiments were devised to show to what extent, if at all, the sporanges on the right half of the culture are discharged at the left hand opening, and *vice versa*.

The apparatus used was a wooden box 39 centimeters long, 23 centimeters wide and 16 centimeters high. Two openings two centimeters square were cut in one end, eight centimeters apart and four centimeters from the bottom of the box. The culture lay on the bottom of the box, its surface vertical and facing the end containing the openings and its center equidistant from the openings. The distance from the center of the culture to the center of either opening was ten centimeters, making the angle between the two beams of light reaching the culture about 45°. The sporanges were caught on two plates of glass. One, as usual, was placed inside against the end of the box (covering the openings) and the other was placed vertically, perpendicular to the first glass at its median line and extending back until it touched the median line of the culture. Figure 16 is a diagram of a horizontal cross section of the box. With this arrangement, any sporanges discharged from the left half of the culture towards the right opening or from the right half to the left opening will be intercepted by the second glass.

Three experiments were performed with the culture ten centimeters from the openings. In the first experiment (Table XII), 21 out of 126 sporanges were intercepted by the second glass; in the second, 19 out of 144; and in the third, 11 out of 99. The average thus intercepted in the three experiments is 14%.

Three similar experiments were performed with the culture 25 centimeters from the openings. In the first, 40 out 126 were on the second glass (30%); in the second, 31 out of 124 (40%); and in the third, 122 out of 317 (38%). The average percentage for the three experiments is 36, or 22% more than when the culture was 10 centimeters from the openings.

The respective beams of light reaching a given sporangiophore from the two openings do not differ greatly in intensity,

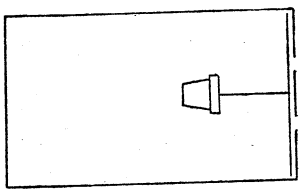

Fig. 16. Diagram of horizontal cross section of box used in table XII.

whatever the position of the sporangiophore in the culture, yet the majority of the sporanges are shot toward the nearer opening, that is, toward the source of the stronger illumination. It is evident that very small differences in light intensity are perceived by the sporangiophores.

TABLE XII.—*Showing to what extent sporanges are thrown to the farther of two simultaneously presented lights.*

	On first glass.	On second glass aimed at right opening.	On second glass aimed at left opening.	Total.	Per cent on second glass.
10 cm. from culture to openings.	105	7	14	126	16.7
	125	10	9	144	13.2
	88	6	5	99	11.1
25 cm. from culture to openings.	86	27	13	126	30.1
	93	18	13	124	40.
	195	63	59	317	38.

When the culture was 25 centimeters from each opening (Table XII), 36% of the sporanges were shot toward the more distant opening, while only 14% were shot toward the more distant opening when the culture was 10 centimeters away. This is easily explained. As the culture is moved further from the openings, being kept equidistant from them, the amount of light from the respective openings reaching any particular sporangiophore not in the median line of the culture becomes more nearly equal. The ratio approaches 1:1 as a limit as the distance increases. When the culture is placed at a considerable distance from the openings, then, sporanges from the whole surface of the culture contribute to the group at each of the openings.

The Response of Pilobolus to the Simultaneous Stimuli of Two Differently Colored Lights.

The relative efficiency of lights of different colors in causing heliotropic reaction in *Pilobolus* has already been considered in connection with the question of accuracy of aim. It was found that the reactions to white and blue lights were the most precise; that those to yellow light were much less so; and that the reactions to red light were very vague and indefinite. The behavior of *Pilobolus* was next studied when subjected simultaneously to lights of two different colors.

The cultures were placed in a box 38 centimeters long, 17 centimeters wide and 23 centimeters high. In the middle of each end was a circular opening two centimeters in diameter. The same blue, ruby, yellow and colorless glass plates were used as in the foregoing experiments, and in addition potassium bichromate and copperoxide solutions contained in parallel-walled flasks. The plates of colored glass were placed one against each opening, precautions being taken to prevent the entrance of light around their edges. When solutions were used, the flat flasks were placed close against the opening and on the outside of the box, black pads encircling the opening ensuring the exclusion of all white light. Against the inside of each end of the box was placed a plate of colorless glass to catch the spor-

anges. Sixteen candle power incandescent lamps were used as sources of light and were kept before the opening day and night. The culture was placed upright in a saucer containing water to prevent it from drying out, and was set on the floor of the box in line with the two openings. The culture was first placed two centimeters from one end of the box, and on each succeeding day was moved two centimeters toward the opposite end. The number and position of the sporanges fired while the culture was in each position were recorded. The surface of the culture was six centimeters below the level of the centers of the two openings. The angles at which the light from the respective openings strikes the sporangiophores vary with the position of the culture. To obviate the possible shading of some of the sporangiophores, care was taken to use cultures with an even surface and with sporangiophores symmetrically arranged.

As the culture is moved from one end of the box toward the other, the intensity of the light received from one opening is of course decreased while that from the other is proportionally increased. By recording the number of sporanges found on each glass at each stage in the series, we can determine the ratio of the effectiveness of the two kinds of light.

In the experiments to be described, we compared the effects of light coming through respectively (1) the blue and the white glass, (2) the blue and the yellow glass, (3) the ammoniacal copper oxide solution and the bichromate solution, (4) the blue and the red glass, and (5) the white and the red glass.

In the series in which the blue and white glasses were used, the bull's eye target, Fig. 3, was used, the inner circle being of the same size as the opening. The radius of each circle was one centimeter greater than that of the circle next within. The areas were numbered outward from 1 to 3, beginning with the inner circle. Area 3 included all outside of area 2. More circles were unnecessary, since relatively few sporanges were found outside of area 2. A horizontal line was drawn through the center of area as before, so that a record could be made of the sporanges striking above and below the center. In these preliminary experiments, no attempt was made to compare ab-

solutely the intensity of the two lights used, the object being to determine the relative effect of the colors with varying intensity due to distance.

When the culture was two centimeters from the blue glass and 36 centimeters from the white, 88.6 per cent of the sporanges were thrown toward the blue, 11.4 per cent toward the white. When the culture was eight centimeters from the blue and 30 centimeters from the white, 74.8% and 25% were found on the two glasses respectively. At 12 centimeters from the blue and 26 centimeters from the white, 52.8% struck the blue glass and 47.1% the white, the numbers now being almost equal although the culture is still less than half as far from the blue glass as from the white. When the culture was at 18 centimeters from the blue and 20 centimeters from the white, only 30% struck the blue and 70% the white. At 28 centimeters from the blue and ten centimeters from the white, all the sporanges were fired toward the white end. Table XIII contains the data in complete form. It is seen that in no case, even when the ratio was 2 to 36 in favor of the blue, were all the sporanges discharged toward the blue. The curve in Figure 17 is plotted from these data. The percentages of sporanges fired toward one of the two glasses are used as abscissas and the distances from the culture as ordinates. The area above the curve indicates the proportion which struck the white glass and the area below, that which struck the blue glass. Although the

TABLE XIII.—*Blue glass and white glass at opposite ends of the box. Culture moved 2 cm, each day from 2 cm. from the blue glass to 2 cm. from the white glass. Opening 2 cm. in diameter.*

Distance from blue.....	2 cm.	4 cm.	6 cm.	8 cm.	10 cm.	12 cm.	14 cm.	16 cm.	18 cm.
Number on blue.....	101	50	95	218	20	101	237	25	10
Per cent on blue.....	88.6	89.2	90.49	74.82	71.42	52.87	62	37.92	30.3
Distance from white....	36 cm.	34 cm.	32 cm.	30 cm.	28 cm.	26 cm.	24 cm.	22 cm.	20 cm.
Number on white.....	13	6	10	5	8	90	135	36	23
Per cent on white.....	11.4	10.8	9.52	25.17	28.57	47.12	38	62.07	69.69
Distance from blue.....	20 cm.	22 cm.	24 cm.	26 cm.	28 cm.	30 cm.	32 cm.	34 cm.	36 cm.
Number on blue.....	3	2	1	11	0	13	0	1	0
Per cent on blue.....	1.61	5.88	3.03	5.55	0	4.58	0	.96+	0
Distance from white....	18 cm.	16 cm.	14 cm.	12 cm.	10 cm.	8 cm.	6 cm.	4 cm.	2 cm.
Number on white.....	183	32	32	75	142	231	222	142	190
Per cent on white.....	98.38	94.11	96.96	94.44	100	95.41	100	99.03	100

percentages vary irregularly, it is noticeable that on the whole there is a gradual decrease in the proportion fired toward the blue and a proportionate increase in that fired toward the white as the culture is moved toward the latter. It appears then that the light of short wave length, as such, has no preponderating influence at least in determining the phototropic reactions of *Pilobolus.*

Whether the presence of two sources of light will affect the accuracy of aim of the sporangiophores can be ascertained from the results of the experiments just described by studying the number of sporanges which strike in the different areas of the respective targets. It would seem possible that if the presence of one light influences the aim of the sporangiophores toward the other, some of the sporangiophores should be pulled out of the position of direct aim and nearer the vertical position. If, then, there be such an influence as that suggested, we shall except in these experiments to find a larger percentage of the sporanges above the center of the target than in the case in which only one light was used.

We note, however, that the majority of the sporanges are always below the center of the opening. On the blue glass there are 28% more below than above with the culture at four centimeters distance, 49.3% more at 14 centimeters, 45.6% more at 26 centimeters distance. In the case of the white light, also, the number below always exceeds that above the center of the opening. These results are given in complete form in Table XIII. The results make it seem probable that there is no increase in the number of sporanges landing above the opening due to the presence of a second source of light.

A possible effect might also be shown in a general tendency to inaccuracy of aim toward one light due to the presence of the other. The percentages of the total number of sporanges which land in area 1 in the blue and white lights respectively, are, however, practically the same as in the case in which the two glasses were used separately (Compare Table XV with Table II). As before, there is a slightly larger proportion on area 1 when the blue light is used than when the white light is used.

TABLE XIV.—*Culture at distances indicated. Opening 2 cm. in diameter. Blue and white glasses at opposite ends of box.*

On blue glass.	2 cm.		4 cm.		6 cm.		8 cm.		10 cm.		12 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
Above	26	25.7	18	36.	36	37.8	67	30.7	8	40.	41	40.6
Below	75	74.2	32	64.	59	62.1	151	69.2	12	60.	60	59.4
Difference	—49	—48.5	—14	—28.	—23	—24.3	—84	—28.5	—4	20.	—19	—8.8
On white glass.												
Above	13	36.9	92	35.2	58	26.	93	44.4	62	43.	82	46.
Below	177	63.1	142	64.8	164	73.9	128	55.6	80	57.	93	54.
Difference	—154	—56.2	—50	—29.6	106	47.9	—35	11.2	—18	—14.	—11	—8.
On blue glass.	14 cm.		16 cm.		18 cm.		20 cm.		22 cm.		24 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
Above	60	25.3	11	44.	3	30.	1	33.3	0	0.	0	0.
Below	167	74.6	14	56.	7	70.	2	66.6	2	100.	1	100.
Difference	107	—49.3	—3	—14.	—4	—40.	—1	—33.3	2	—100.	—1	—100.
On white glass.												
Above	15	46.9	16	50.	72	39.3	9	39.1	9	25.	53	39.2
Below	17	53.1	16	50.	111	60.6	14	60.8	27	75.	82	60.7
Difference	2	6.2	0	0.	—39	—21.3	—5	—21.7	—18	—50.	—29	—21.5
On blue glass.	26 cm.		28 cm,		30 cm.		32 cm.		34 cm.		36 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct	No.	Per ct.
Above	3	27.2	0	0.	0	0.	0	0.	0	0.	0	0.
Below	8	72.8	0	0.	0	0.	0	0.	0	0.	0	0.
Difference	—5	—45.6	0	0.	0	0.	0	0.	0	0.	0	0.
On white glass.												
Above	35	43.3	2	25.	25	33.3	0	0.	2	33.3	5	38.4
Below	51	56.6	6	75.	50	66.6	10	100.	6	66.6	8	61.6
Difference	—16	—13.3	—4	—50.	—25	33.3	—10	—100.	—4	33.3	—3	22.2

TABLE XV.—*Culture at distances indicated. Opening 2 cm. in diameter. Blue and white glasses at opposite ends of the box. Table shows number of sporanges on the different areas with the cultures at the various distances.*

	2 cm.		4 cm.		6 cm.		8 cm.		10 cm.		12 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
Blue glass—1.....	31	30.6	44	88.	80	84.2	107	49.	12	60.	55	54.4
Blue glass—2.....	42	41.5	5	10.	8	8.4	75	34.4	4	20.	32	31.6
Blue glass—3.....	28	27.7	1	2.	7	7.3	36	16.9	4	20.	14	13.8
Total.....	101	100.—	50	100.	95	100.—	218	100.	20	100.	101	100.
White glass—1.....	103	54.2	100	70.4	143	44.4	166	76.1	112	78.8	77	44.
White glass—2.....	82	43.1	20	14.	58	18.	44	19.	24	16.8	62	35.4
White glass—3.....	5	2.6	22	15.4	121	37.5	21	.9	6	4.1	36	20.5
Total.....	190	100.	142	100.	322	100.	231	100.	142	100.	175	100.—

	14 cm.		16 cm.		18 cm.		20 cm.		22 cm.		24 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.	No.	Per ct.
Blue glass—1.....	156	65.8	9	36.	7	70.	2	66.6	2	100.	1	100.
Blue glass—2.....	33	13.8	11	44.	1	10.	1	33.3	0	0.	0	0.
Blue glass—3.....	48	20.2	5	20.	2	20.	0	0.	0	0.	0	0.
Total.....	237	100.	25	100.	10	100.	3	100.	2	100.	1	100.
White glass—1.....	25	78.1	26	81.2	62	33.9	9	39.1	13	36.1	42	31.1
White glass—2.....	6	18.7	3	9.3	68	37.3	6	34.7	11	30.5	33	24.1
White glass—3.....	1	3.1	3	9.3	53	28.8	8	26.	12	33.3	61	45.1
Total.....	32	100.	32	100.	183	100.	23	100.	36	100.	135	100.

TABLE XV.— *Culture at distances indicated. Opening 2 cm. in diameter. Blue and white glasses at opposite ends of the box. Table shows number of sporanges on the different areas with the cultures at the various distances*—Continued.

	26 cm.		28 cm.		30 cm.		32 cm.		34 cm.		36 cm.	
	No.	Per ct.	No.	Per ct.	No.	Per ct	No.	Per ct.	No.	Per ct.	No.	Per ct.
Blue glass—1.	11	100.	0	0.	5	38.4	0	0.	1	100.	0	0.
Blue glass—2.	0	0.	0	0.	3	23.2	0	0.	0	0.	0	0.
Blue glass—3.	0	0.	0	0.	5	38.4	0	0.	0	0.	0	0.
Total	11	100.	0	0.	13	100.	0	0.	1	100.	0	0.
White glass—1.	38	42.2	2	25.	21	28.	4	40.	2	33.3	2	15.3
White glass—2.	35	38.8	3	37.5	30	40.	0	0.	2	33.3	5	37.6
White glass—3.	27	30.	3	37.5	24	32.	6	60.	2	33 3	6	46.1
Total	90	100.	8	100.	75	100.	10	100.	6	100.	13	100.

In our next series of experiments, the blue glass was placed at one opening and the yellow glass at the other. With the culture two centimeters from the yellow glass more than half of the sporanges were fired toward the blue, although it was eighteen times as far away as the yellow light. With the culture in the middle of the box, 91% were discharged toward the blue, and at 22 centimeters from the yellow light all the sporanges were fired toward the blue. The decrease in the percentage of sporanges reaching the yellow glass was very irregular, probably because of inequalities in the distribution of the sporanges on the surface of the cultures. The exact data for each observation are given in Table XVI. The curve (Figure 18) is plotted from these data and shows the proportion of sporanges fired toward the blue and yellow glasses respectively.

TABLE XVI—*Culture at distances indicated. Opening 2 cm. in diameter. Blue and yellow glasses at opposite ends of the box.*

Distance from blue	4 cm.	6 cm.	8 cm.	10 cm.	12 cm.	14 cm.
Number on blue	54	36	18	10	28	177
Per cent. on blue	100	100	100	100	100	100
Distance from yellow	34 cm.	32 cm	30 cm.	28 cm.	26 cm.	24 cm
Number on yellow	0	0	0	0	0	0
Per cent. on yellow	0	0	0	0	0	0
Distance from blue	16 cm.	18 cm.	0 cm.	22 cm	24 cm.	26 cm.
Number on blue	20	80	211	72	52	80
Per cent. on blue	100	88.2	91.34	75.78	96.29	95.24
Distance from yellow	22 cm.	20 cm.	18 cm.	16 cm.	14 cm.	12 cm.
Number on yellow	0	4	20	23	2	3
Per cent. on yellow	0	11.7	8.65	24.21	3.70	4.76
Distance from blue	28 cm.	30 cm.	32 cm.	34 cm.	36 cm.	
Number on blue	38	29	61	19	55	
Per cent. on blue	100	63.04	68.54	70.37	55.64	
Distance from yellow	0 c m.	8 cm.	6 cm.	4 cm.	2 cm	
Number on yellow	0	17	28	8	69	
Per cent. on yellow	0	36.95	31.46	29.62	44.35	

As in the experiments with blue and white lights, the accuracy of aim toward either the blue or the yellow did not seem to be affected by the presence of the other color.

We next tried the effect of placing the copper sulphate and bichromate solutions at opposite ends of the box. On comparison of the results with those when the blue and yellow glasses were used, a difference is found in that at two centimeters from the bichromate solution only a small percentage of the

sporanges were discharged toward the blue, while when the glasses were used over half went toward the blue. Furthermore, the percentage fired toward the yellow solution decreases more regularly as the culture is moved toward the blue, although on the whole there are about the same percentages on the blue and yellow respectively as when the glasses were used. The detailed results are given in Table XVII. Figure 21 shows the proportion of sporanges fired toward each of the two solutions.

TABLE XVII.—*Culture at distances indicated. Opening 2 cm in diameter. Blue and yellow solutions at opposite ends of the box.*

Distance from yellow	2 cm.	9 cm.	12 cm.	19 cm.
Number on yellow	41	15	12	8
Per cent on yellow	91.1	30	17—	3.6
Distance from blue	6 cm.	29 cm.	25 cm.	19 cm.
Number on blue	4	35	59	214
Per cent on blue	8.9	70	83+	96.4

Next the red and white glasses were placed at opposite ends of the box. With the culture two centimeters from the red and 36 centimeters from the white, 85% of the sporanges were discharged toward the red. At four centimeters, only one-fourth were sent toward the red. At 10 centimeters from the red, all were sent toward the white. Table XVIII contains the complete data for this series of experiments. Taking the sum of the results of this series of experiments, twelve times as many sporanges all told were fired toward the white as toward the red light (Figure 20). The accuracy of aim, as shown by the sporanges which hit the opening with the red light, was very poor, just as was the case when the red light was used alone. There is no indication that one light affected the accuracy of aim of the sporangiophores toward the other.

TABLE XVIII.—*Culture 2–16 cm. from red. Opening 2 cm. in diameter. Red and white lights at opposite ends of the box.*

Distance from red	2 cm.	4 cm.	6 cm.	8 cm.	10 cm.	12 cm.	14 cm.	16 cm.
Number on red	66	13	1	1	0	0	0	0
Per cent on red	85.6	29.5	3.5	2.5	0	0	0	0
Distance from white	36 cm.	34 cm.	32 cm.	30 cm.	28 cm.	26 cm.	24 cm.	22 cm.
Number on white	11	31	28	30	40	68	64	33
Per ct on white	14.4	70.4	96.5	97.5	100.	100.	100.	100.

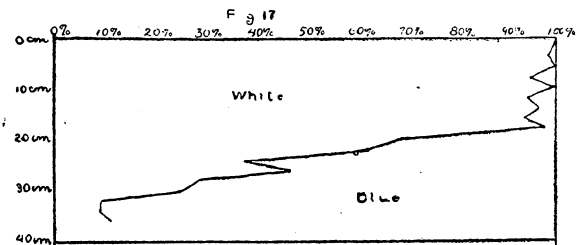
Fig 17
0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
0cm
10cm
20cm
30cm
40cm
White
Blue

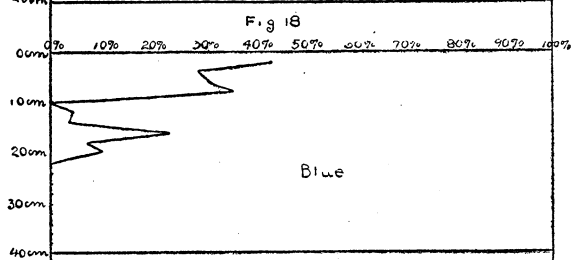
Fig 18
0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
0cm
10cm
20cm
30cm
40cm
Blue

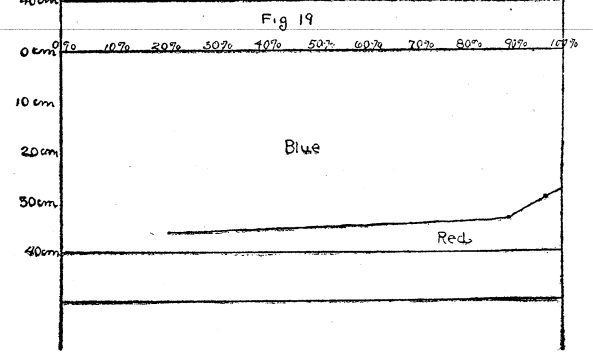
Fig 19
0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
0cm
10cm
20cm
30cm
40cm
Blue
Red

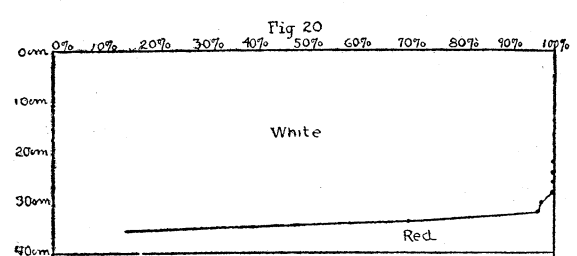
Fig 20
0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
0cm
10cm
20cm
30cm
40cm
White
Red

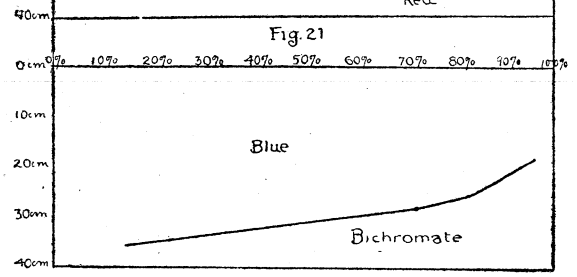
Fig. 21
0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
0cm
10cm
20cm
30cm
40cm
Blue
Bichromate

Similar experiments were tried, using the blue and red glasses. Only at two centimeters from the red did a majority of the sporanges reach the red glass. At ten centimeters, none were fired toward the red. Table XIX gives the data for these experiments. As in the case of the red and white lights, twelve times as many sporanges all told were fired toward the blue as toward the red light (Figure 19).

As in the preceding cases, the accuracy of aim toward either light appears to be unaffected by the presence of the other.

TABLE XIX.—*Culture at distances indicated. Openings 2 cm. in diameter. Blue and red lights at opposite ends of the box.*

Distance from red	2 cm.	4 cm.	8 cm.	10 cm.
Number on red	68	10	1	0
Per cent on red	78.1	11.5	4	0
Distance from blue	36 cm.	34 cm.	30 cm.	28 cm.
Number on blue	19	77	27	41
Per cent on blue	21.8	88.5	96+	100

GENERAL DISCUSSION

The wide upper part of the top-shaped vesicular bulb of *Pilobolus* is broader than the sporange which caps it; consequently, when the sporangiophore is pointing directly toward a small source of light, only the sporange and a narrow rim around the upper end of the vesicular bulb are exposed to direct illumination. The slender stalk below the bulb under such circumstances is shaded.

Steyer (21) has obtained evidence that in *Phycomyces* the sensitive and motor zones in the sporangiophore coincide and are located just below the sporange. In *Pilobolus,* after sporange and vesicular bulb are formed, the motor zone is in the upper end of the sporangiophore, just below the vesicular bulb. If, as in *Phycomyces,* the motor is also the sensitive region, the final adjustment in the process of aiming, that is, after the sporangiophore has come to point almost directly toward the light, is not made under the influence of direct illumination; for such light as reaches this portion of the stalk must have been

reflected backwards inside the bulb. It may be, however, that the bulb itself is sensitive and transmits the stimulus to the motor zone below. In either case the perfect symmetry of the bulb probably facilitates exact aiming, for a one-sided bulb would be unequally illuminated on its different sides.

Sensitiveness to light, as our experiments show, is maintained throughout the later stages of development; and in spite of the changing turgor within the bulb and the sporangiophore immediately preliminary to the discharge of the sporange, even at this late stage an accurate heliotropic reaction can be carried out. The two sets of processes, leading respectively to the discharge of the sporange and to a change of aim on the part of the sporangiophore, proceed independently of each other, neither inhibiting the other. If Noll's theory that curvature is due to an unsqual change in the elasticity of the walls of a cell, be extended to apply to the present case, it must be assumed that there is a very nice adjustment of the amount of change in elasticity as the turgor of the bulb increases. With one degree of turgor, a given increase in elasticity of the wall on one side of a sporangiophore would result in a stretching of the wall on that side and a consequent bending of the cell as a whole. With a greater degree of turgor, an equal change in elasticity would result in a greater stretching and so in a greater curvature. Yet we find that reactions are essayed and successfully controlled during the period of the final turgor changes within bulb and sporangiaphore. It would be interesting to know whether reactions are performed more rapidly when the turgor is greater, but this is a question which we have not yet investigated.

Whatever the mechanism in *Pilobolus* for the perception of light may be, it is certainly efficient. For example, in the white light, ninety-five per cent of the sporanges struck a four-centimeter opening when the culture was twenty centimeters distant from the light; and with one or two exceptions, the remaining five per cent struck within one or two centimeters of the opening. It is plain that the aiming has been done with remarkable precision.

The experiments in which a single white light was used afford some evidence as to the factors which influence the distribution of the sporanges on the receiving plate. These factors naturally fall into two classes: those which concern the structure and operation of the aiming and firing mechanism, and those which are external to the individual sporangiophore.

Nearly all of the sporanges struck the glass immediately over the opening through which light was admitted, when the distance from culture to opening was relatively short. Even in those cases, a few sporanges did not reach the opening but struck below, above or to one side. There is therefore some variation in the accuracy with which the individual sporangiophores aim toward the source of light. This difference may result from an unequal sensitiveness of perception on the part of individual sporangiophores, or from a difference in the perfection of the firing apparatus, or from a combination of both causes. It is probably to be traced back to some factors in the organism itself rather than to external conditions at the time of discharge. Whatever may be the cause of this variation in the accuracy of aim, it is apparent that it has an important bearing on the final results in all of the experiments.

Not only is there a variation in the accuracy with which the individual sporanges are fired toward the light, but the force with which they are discharged is plainly quite unequal. Even at comparatively short distances, as we have seen, some of the sporanges fell below the opening; and at the greater distances individual difference in this respect stood out much more plainly. Some of the sporanges struck the opening even when the culture was at a distance of ninety-two centimeters, although sixteen and seven-tenths per cent failed at this distance to reach the vertical glass and fell on the floor of the box. Variations in the force of the discharge, therefore, as well as in accuracy of aim are to be reckoned with in the consideration of the final results.

Our experiments have made it plain that the distance of the culture from the source of light is another deciding factor in the distribution of the sporanges. The number of spo-

ranges falling upon the opening decreases with an increase in the distance of the culture from the source of light (as shown by Table I), because of the effect produced by gravity upon the path of the flying sporange.

The arrangement of the sporangiophores in the culture is also to be taken into account, since, as we have pointed out and as Figures 8 and 9 plainly show, this factor may be a source of irregularity in the final distribution of the sporanges upon the glass plate—since it of course affects the position of the individual sporangiophore relatively to the source of illumination.

It was found that the proportion of sporanges which reached the opening varied somewhat with the size of the latter, the proportion being greater with the larger opening. Although no extensive series of experiments was performed with reference to this point, the results obtained were sufficiently decisive to show that the size of the opening is a factor which affects the accuracy of aim of the sporangiophores.

There are doubtless other factors which influence the final distribution of the sporanges, but upon which our experiments have as yet thrown no particular light. Light intensity is certainly one such factor, and another, to be discussed below, is the wave length of the light which serves as a stimulus. But when all of the factors are taken into consideration which may influence the path taken by the sporanges and their ultimate position, it becomes evident from our observations as well as from those of previous investigators that the sporangiophores of *Pilobolus* aim with remarkable accuracy toward a source of white light.

Toward a blue light the sporangiophore is aimed, on the whole, just about as accurately as toward a white light. In some cases, indeed, the reaction is even more accurate; for example, when the culture was twenty centimeters from a four-centimeter opening, ninety-seven per cent of the sporanges fell upon the opening.

Toward a yellow light, on the other hand, we find that the aim is much less accurate than toward either the blue or the

white; and the red light seemed to be the least efficient of all in producing accuracy of aim. In fact, there seemed to be almost no definiteness of aim whatever in the cultures subjected to the red light. So far as we can find, no explanation has yet been suggested for the different efficiencies of lights of different colors in cases of this sort.

The question as to whether the sporangiophore of *Pilobolus* is aimed high in order to make allowance for the distance the sporange must travel to reach the light, appears to be settled by the data given in Table VII. The sporangiophore is aimed straight toward the light, making no allowance for distance. The sporange follows the path of a projectile, and the distance that it falls below the source of illumination increases with an increase in the distance it must travel. The curve (Figure 11) which represents the results of our observations upon this point, shows the average path of a large number of sporanges. Any individual sporange, as just pointed out, is fired straight toward the opening, and its path is that of a projectile, since gravity tends to pull it downward as is the case with any body thrown horizontally or approximately so. Different sporanges, as has been shown, vary in the accuracy with which they are aimed and in the force of their discharge; and these individual differences necessarily influence the direction of the curve. Consequently, the curve (Figure 11) which shows their average path does not represent the path of any single sporange and is therefore not a parabola.

Nathansohn (14) in testing Talbot's law for plants, found the point between a constant and an intermittent light at which the two had equal power to induce a heliotropic reaction in seedlings of *Brassica*. It is noteworthy that at this point, where the difference between the two lights (as tested by the human eye) was less than one per cent, each seedling reacted accurately to *one* light only. About half of the seedlings experimented with, bent toward one light, the other half toward the other light. In other words, one light in each case produced a full and complete reaction and the other had no visible effect. Which of the two lights would prevail could

not be predicted. In a few cases in which *Avena* seedlings were used, no reaction occurred. On the other hand, with *Brassica,* the most sensitive of the plants worked with, there was always a reaction in one direction or the other.

Pilobolus also, as our experiments show, when subjected to two approximately equal beams of light, aims and discharges at one or the other of the two. In the majority of cases, the aim toward one source of light is as accurate as though the other were not in existence. Light rays from both sources reach the sensitive sporangiophore. Apparently there is nothing to prevent each set of light rays—or each individual light ray for that matter—from setting up those changes in the protoplasm which constitute the perception of a stimulus, and nothing to prevent these simultaneous stimuli from acting together to produce a resultant reaction. But this does not occur. The visible reaction of each sporangiophore is to one and one only of the two possible sources of stimulation.

When the two openings which serve as sources of illumination are close together, there are, to be sure, a small number of sporanges which land about midway between the openings. Attention has already been called to the fact that these particular sporanges often fall considerably below the level of the rest of the sporanges on the glass. When the openings are so far apart that the angle between the two beams of light is more than 20°, the sporanges which fall markedly below their fellows are found, not under the middle of the field, but beneath one or the other of the two openings.

It is possible that the sporanges which fall between and below the openings came from sporangiophores which perceived and reacted to both lights at once, thus aiming at a point between the two openings. But if this be the case, why should the resultant reaction to two simultaneous stimuli appear only when the openings are close together? If two separate beams of light can be perceived at the same time by a single cell, and if each separate perception can produce its influence in the motor region, thus giving rise to a true resultant reaction, it is hard to see why the feat is not performed at least as often

when the angle between the two sources of light is 40° as it is when the angle is 15°. On this assumption, also, why should there not be a similar resultant reaction when the two stimuli are of unequal intensity? The lesser stimulus would be expected to produce a smaller effect, but why should it not exercise a proportional influence upon the reaction instead of being entirely neglected by the sporangiophore, as we have found is the case? It remains unexplained, too, on the assumption of a simultaneous influence of the two stimuli, why the sporanges which land in the median region usually lie at a lower level than do those which are aimed at one or the other of the two openings.

The facts therefore suggest strongly that the sporangiophores which produced these particular sporanges were like the less sensitive *Avena* seedlings used by Nathansohn. They were probably weak and imperfect and lacked not only the turgor to give force to the discharge, but also the sensitiveness to perceive in two neighboring lights anything but one general source of illumination.

What factors determine, when two lights are presented, which of the two lights shall prevail, is not always apparent. A slight difference in the relative intensity of the two lights may make a marked difference in the proportional numbers of sporanges found at the respective openings. This was well shown in the experiments in which the sporanges fired from the left half of the culture to the right opening and from the right half to the left opening were intercepted upon a vertical glass perpendicular to the culture surface. When the culture was ten centimeters from the openings, 14 per cent of the sporanges were caught upon this glass (Table XII). When the culture was 25 centimeters from the openings, 36% were caught upon it. The alteration in the relative intensity of the light received from the two sources was not great, but it made a difference in the result of 22 per cent of the total number of sporanges discharged.

Again, the sporangiophores in a culture are not always perpendicular to its surface when the experiment is set up. Con-

sequently, the light rays from the two openings do not hit a given sporangiophore at the same angle. We have not determined for *Pilobolus* the angle at which the incident light rays produce maximum stimulation, but it is very probable that the efficiency of a given light does vary with the angle at which it strikes the sporangiophore. This, too, may aid one set of light rays in gaining dominance.

Besides these factors, there are doubtless conditions within the organism itself which we are at present unable to define, that may determine or aid in determining which light shall produce a visible effect. It is not impossible, for example, that different sides of the same sporangiophore may vary in sensitiveness, and that this difference in sensitiveness may decide the course of the reaction.

The experiments dealing with the response of *Pilobolus* in the presence of two differently colored lights involve much the same problems that we discussed in connection with the experiments in which two white lights were used simultaneously. The data (Table XIV) showing that the presence of one light does not affect the aim of the sporangiophore toward a second light seem to corroborate the results obtained in the case in which the two white lights are used simultaneously. For example, with the blue and white glasses placed at opposite ends of the box, there are found on the blue glass twenty-eight per cent more below than above the center of the opening when the culture is four centimeters distant, forty-nine and three-tenths per cent more at fourteen centimeters, and forty-five and six-tenths per cent more at twenty-six centimeters. In the same way, the number below the opening on the white glass always exceeds that above. There is no apparent change of aim towards one light due to the presence of the second light, for this would result in a larger proportion of the sporanges striking above the center of one of both openings. The number of sporanges below the center of the opening always exceeded that above the center, just as was the case when a single light was used, and the proportional excess below the center was about the same.

A comparison of the reactions of *Pilobolus* toward each of two colored lights used simultaneously gives results in harmony with those obtained when similar lights were used separately. The reaction is most accurate toward the blue light when the latter is used in combination with the white, the yellow or the red. A larger percentage of the sporanges strike the glass at the opening in the blue light. The reaction toward the white light is a little less accurate than that toward the blue, as is shown when the white light is used simultaneously with the blue, red and yellow. A comparison of the average percentages reaching the respective openings in the whole series of experiments (Table XV) shows plainly that a larger percentage strikes the opening in the blue than in the white light. The average percentage striking the opening in the blue light was 57.9; that in the white light 47.3. The reaction toward the yellow light is much less accurate than that toward either the blue or white, while the reaction toward the red light is the least accurate of all.

We have found that when the culture is exposed to blue and white lights simultaneously, the ratio of the total number of sporanges fired toward the respective lights is 4 to 7 in favor of the white light, although, as our previous experiments, just cited, had shown, the sporangiophores are aimed, if anything, rather more accurately toward a single blue light than toward a single white light. The results with the simultaneous blue and white lights seem to show that the relative intensity of the light is a prime factor in determining the reaction. The intensity of the white light used is greater than that of the blue, since the blue glass cuts out a large portion of the spectrum, thereby diminishing the intensity of the light. It must be left to further investigations to determine whether the number of sporanges fired respectively toward the simultaneously acting blue and white lights is proportional to the relative intensities of the two lights.

The ratio of the total numbers of sporanges fired respectively toward simultaneous blue and yellow lights was 9 to 1 in favor of the blue. These results are in harmony with those ob-

tained in the experiments with the single lights of different colors, in which it was found that, as in the present case, the sporanges were aimed much more accurately toward the blue than toward the yellow light.

When either white or blue light was used simultaneously with red, the ratio was 12 to 1 in favor of the white or blue light. This also agrees with the results of the previous experiments which tested the accuracy of aim of *Pilobolus* toward single lights.

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ON THE CYTOLOGY AND EMBRYOLOGY OF SMILACINA RACEMOSA,

BY FREDERICK MC ALLISTER

The conception of the constancy of the chromosome number in the nuclei of individuals of different species and the numerical equality of the paternal and maternal chromosomes in diploid nuclei as established by Flemming (28), Strasburger (92), Guignard (33, 34), Van Beneden (4), Boveri (12, 13) and their contemporaries, underlies all recent studies of reduction phenomena in plants and animals. That this constancy of the chromosome number is also the starting point for all studies of the individuality of the chromosome is self evident. As has been pointed out by Harper (39), the constant recurrence of a characteristic number of chromosomes in the nuclei of the organism during mitosis is nevertheless alone insufficient to establish the individuality of the chromosomes. Rabl's idea (78) of the reorganization of the chromosomes from the resting reticulum, involved the material as well as the numerical persistence of the chromosomes.

Boveri's earlier work on Ascaris (10, 11, 13) demonstrated that the same number of chromosomes reappears in the prophases as had disappeared in the reticulum in the preceding telophase, and further that they reappeared in the same position in which they had disappeared.

The discovery by Henking (40) of a permanent difference in the form and size of certain chromosomes in the nuclei of various species, indicating thus a qualitative difference among chromosomes, has given considerable further support to the doctrine of the permanence of the chromosomes. The accessory

chromosomes found in the nuclei of various insects by Henking (40), Montgomery (60, 61), Sutton (101), Paulmier (76), de Sinety (92) and others, have been shown to be as constant nuclear elements as the ordinary chromosomes.

For plants the work of Strasburger (95, 96), Miyake (55), and Miss Sykes (102) on Funkia, of Rosenberg (79) on Listera ovata, of Miyake (55) on Galtonia and of Müller (68) on Yucca has shown that a constant difference in the size of the chromosomes exists in these species. Müller further reports that the small chromosomes in Yucca lag behind in the anaphase and go into the resting condition on the side of the daughter nucleus toward the equatorial plate and that in the following prophase they reappear in the same position from which they had disappeared.

More recently the discovery of the so-called prochromosomes of Rosenberg and Overton in the nuclei of certain species has given no little support to the doctrine of the individuality of the chromosome. These bodies occur in the nuclear reticulum as persistent conspicuous chromatic masses of the same number as the chromosomes and vary from a nearly spherical form in some of the Cruciferae to an elongated rod-like structure in Thalictrum and Calycanthus. The persistence of these centers about which the chromatin of the reticulum is aggregated in the prophases has enabled Overton (74) to follow the course of the chromosomes of Thalictrum and Calycanthus through the reduction divisions and to observe that these centers, at least, retain their identity throughout. Overton has reviewed the literature of this subject fully.

Fick (27) regards the chromosomes as not persisting from one cell generation to the next but changing in composition as the ranks of a maneuvering army may change from day to day without changing the composition of the army.

The work of Häcker (36) and of Rückert (81) on Cyclops demonstrated that in these forms there is no fusion of the paternal and maternal chromosomes in fertilization. For a time at least the two sets of chromosomes remain separate and distinct and appear as separate groups in metaphase. Their work has

received striking confirmation in the work of Moenkhaus (56) on hybrids of Fundulus heteroclitus and Menida notata. He found that the paternal and the maternal chromosomes have very distinctive forms and sizes and thus can be traced in the fertilization and the early cleavage stages. They were seen to form distinct groups through the first and second cleavage divisions of the fertilized egg, thus retaining their number, form and relative position, for a time at least.

The cases in which the chromosomes of the male and female nuclei are fully prepared for division at the time of the approximation of the pronuclei in the egg form the most frequent type of fertilization in animals. This is the case in Ascaris, Cyclops, Nereis and a large number of other forms.

For plants the fusion of the sexual nuclei has been commonly reported as taking place in the resting condition,—the resulting fusion nucleus also remaining for a time in the resting condition. Prominent exceptions to this general contention are reported for the Gymnosperms. Here Blackman's observations (8) of two distinct groups of chromosomes in the fusing nuclei in the fertilized egg of Pinus have been confirmed by Chamberlain (15) and especially by Miss Ferguson (26) who found two groups of chromosomes in the spindle of the first division of the fertilized egg. Woycicki (109) found similar conditions in Larix and Murrill (70) in Tsuga, while more recently Nichols (71) has described two chromosome groups in the fertilized egg of Juniperus. Miss Pace (75) reported that in Cypripedium the sexual nuclei had fully formed spirems before they came in contact in the egg. This, as far as I am aware, is the only reported case among the Angiosperms of the fusion of the parental nuclei in any other than the reticulate condition.

We may summarize these evidences as to the individuality of the chromosome as follows: *(1) evidences,—of the constancy of the number of chromosomes, (2) of the numerical equality of the paternal and maternal chromosomes in diploid nuclei, (3) of the reappearance of the same number of chromosomes in the prophases and in the same position as they disappeared in*

the preceding telophase, (4) of the constant recurrence of chromosomes of a characteristic size and shape, (5) of the persistence of chromatic centers of the same number as the chromosomes, and (6) of the independence of the paternal and maternal chromosomes following fertilization.

Harper (39) has pointed out that the doctrine of the permanency of the chromosome does not necessarily imply that the chromosomes themselves are composed of differentiated structures as a cell is, nor that they are elementary independent organisms as Boveri has conceived them to be. The chromosome is to be regarded as a structure "undergoing continually its own series of cyclic changes in resting stage, mitosis, fusion, etc. . . . doubtless susceptible to minor alterations due to changing environment."

The individuality of the chromosome is questioned by some who regard the chromomere as the persistent unit. Mottier (64, 65) is of the opinion that the chromosome does not maintain complete individuality from one cell generation to the other. He believes "that in synapsis or earlier the pangens of like affinity, or those bearing like characters are brought together in chromomeres. These chromomeres are then organized into chromosomes. Each chromosome is accordingly composed chiefly of homologous chromomeres which become arranged end to end, and which split to form paired chromosomes." He is further of the opinion that these chromosomes are distributed according to the rules of chance or according to the affinity which they have one for the other.

Farmer (25) regards the chromomeres as "the discrete units which are responsible for the production of hereditary characters," and therefore the nuclear element which persists from one cell generation to the next. He believes that the relatively small number of chromosomes in cells offers a fatal objection to the view that they are the primordia of the characters of the adult organism and therefore he has selected the chromomere as the structural unit. He cites an example from Darbyshire, who says that in pure races of Pisum sativum and Pisum arvense there are at least 18 pairs of characters which behave in

the hybrid as allelomorphs. Since in the germ cells of these varieties there are only seven chromosomes it would be necessary that the 18 allelomorphs must occur in seven groups, which Farmer regards as improbable if not impossible. He regards the chromosomes as made up of groups or rows of chromomeres, —"but they would not necessarily represent permanent and persistent structures in the sense that each one is to be looked at as being invariably composed of the same chromomeres."

The possibility of the pairing of paternal and maternal chromosomes to form the heterotypic chromosomes seems to have been first urged by Henking (40) in his attempt to explain the origin of the tetrad chromosomes of Pyrrhocoris.

Montgomery (58) figured a series of stages and described the pairing of the chromosomes in the spermatogenesis of Peripatus balfouri. In 1901 he argued that in Ascaris megalocephala univalens pairing of paternal and maternal chromosomes to form the heterotypic chromosome necessarily follows since in the diploid nuclei there is but one chromosome from each parent. He (60) laid the foundation for the study of the sex chromosome by advancing further evidence based upon studies of Hemiptera to the effect that pairing of parental chromosomes here takes place. In Hemiptera heteroptera two small "heterochromosomes" are present in the division stages of the diploid nuclei. These unite during synapsis to form one double heterotypic chromosome and are separated at the time of the hetertypic metaphase so that each germ cell contains but one "heterochromosome." Evidently then in the fertilized egg one "heterochromosome" must come from the male parent and one from the female and it is these two which pair later. From these observations Montgomery argues that the other double chromosomes of the first division must be formed in the same manner.

Winiwarter (108) in 1901 described paired threads as occurring during synapsis in the nuclei of the mother cells of the rabbit and man. This paired condition he connected with the origin of the heterotypic chromosomes, reasoning that the

most probable explanation of the latter was the side by side pairing of paternal and maternal chromosomes when in the thin spirem condition.

Sutton in 1902 (101) reported that the 22 ordinary chromosomes of Brachystola magna could be separated into eleven pairs, each pair differing from the others as to size. Further, in the spermatids after reduction has taken place a graded series of eleven chromosomes can be seen showing that each germ cell has received one member of each of the eleven pairs. He found that the chromosomes of the oogonia as well as those of the ovarian follicle cells corresponded with those of the spermatogonia. The union of the male and female germ cells would therefore give 22 chromosomes, but only eleven different sizes, —or eleven different pairs. These results Sutton believes confirm Montgomery's conclusions as to the pairing and conjugation of paternal and maternal chromosomes.

As early as 1882 Strasburger reported a great dissimilarity in the size of the chromosomes of the pollen mother cells of Funkia Sieboldiana. Guignard in 1899 called attention to the fact of chromosomes of different size in pollen mother cells of Naias major. Koernicke in 1901 also observed that in the embryo-sac of Yucca filamentosa, the chromosomes differed strikingly from one another. These observers did not at the time connect any particular significance with the described facts.

In 1903 (104) de Vries advanced the view that the parental chromosomes remain distinct through the diploid generation and at a period at the close of the sporophyte generation there must be an intimate association of the homologous chromosomes, after which separation occurs. He assumed a side by side pairing as providing the best means for intimate association, and believed that this association occurs previous to the separation of the halves of the heterotypic chromosomes.

Rosenberg has reported (79) that in the embryo-sac mother cell of Listera ovata there are 32 somatic chromosomes of dissimilar sizes. These pair to form the 16 bivalent heterotypic chromosomes of which five are strikingly larger than the re-

maining eleven. Of the five large ones three are very long and the other two decidedly shorter. The eleven smaller ones show differences in size though this is not conspicuous enough to insure accurate counting. He agrees with Montgomery and Sutton that in synapsis a union of the homologous parental chromosomes in pairs takes place.

Strasburger again in 1900 (96) calls attention to the marked differences in the sizes of the chromosomes in the pollen mother cells of Funkia Sieboldiana. Strasburger (97) and Miyake (55) also refer to this disparity in size of the chromosomes of Funkia, and further call attention to differences in the chromosome sizes in Galtonia candicans. Attention is called to the fact that in advanced prophases of the somatic nuclei of Funkia and Galtonia, chromosomes of similar size could frequently be seen lying close together. These are regarded as homologous parental chromosomes, and the double chromosomes of heterotypic diakinesis and metaphases are conceived to arise from the approximation of the members of the pairs when in the leptonema stage.

Funkia has twelve large chromosomes and about thirty-six very small ones. The reduced number as counted in the heterotypic equatorial plate is given as six large and eighteen small chromosomes. In Galtonia there are twelve large and four small chromosomes in the somatic nuclei. In the equatorial plate of the first reduction division six large and two small chromosomes are present according to Miyake. Strasburger (98) believes that the presence of these pairs of chromosomes at the time of the reduction divisions forces one to the conclusion that one chromosome of each pair is of paternal and the other of maternal origin.

Miss Sykes (102) has confirmed the observations of Strasburger and Miyake on Funkia ovata and Funkia Sieboldiana as to the number of somatic and reduced chromosomes as well as to the pairing of the chromosomes in somatic nuclei.

Müller (68), studying the nuclei of root tip cells of Yucca in Strasburger's laboratory, finds ten large and 44 to 46 small chromosomes. In metaphase, chromosomes of like size are fig-

ured lying near one another side by side to form pairs. This arrangement in pairs is seen more or less distinctly through the anaphases and in early telophases.

The pairing of chromosomes even in resting nuclei has been reported by Overton (74), who has observed prochromosomes in the somatic nuclei of Podophyllum and Calycanthus arranged parallel in pairs and connected by linin intervals. In the resting nuclei of the pollen mother cells of these species this arrangement in pairs is still seen, as well as in the later prophases of the heterotypic division.

Very recently Strasburger (100) has discussed at greater length the subject of pairing of homologous chromosomes. He reports that in chloralized root-tips of Pisum sativum, in those cases in which two diploid nuclei fuse, this fusion nucleus upon division shows the chromosomes arranged in pairs, and that even in those cases of the fusion of four diploid nuclei, the division of the fusion nucleus still shows the paired arrangement instead of a tetraploid arrangement. He concludes from these observations that those affinities which are responsible for the approximation of homologous chromosomes are satisfied with the proximity of two such units.

He further calls attention to conditions in the endosperm nuclei of Galtonia candicans as evidence in support of his conclusion that only homologous chromosomes pair. As mentioned above, the haploid nuclei of Galtonia contain six large and two small chromosomes, and the endosperm nucleus would therefore contain twelve large and four small chromosomes from the female polar nuclei and six large and two small chromosomes from the male nucleus. In polar views of the equatorial plate stage Strasburger believes that the chromosomes are arranged to form six pairs of large and two pairs of small chromosomes and in addition six isolated large and two isolated small chromosomes. (See his Figs. 10 and 11, Pl. VI.) The isolated chromosomes are conceived as remaining separate owing to a lack of homologous chromosomes with which to pair.

Rosenberg (80), investigating hybrids of Drosera longifolia × D. rotundifolia,—the former with 40 somatic chromo-

somes and the latter with 20, found that in late prophases of the heterotypic division of the pollen mother cells ten double chromosomes and ten unpaired single chromosomes were present. Rosenberg explains this distribution by assuming that the ten paternal chromosomes pair with ten from the maternal parent, thus leaving ten female chromosomes unpaired. Unfortunately these hybrids do not set fertile seed, so their conduct during the reduction divisions in succeeding generations could not be traced.

Gates (30) has reported, on the other hand, that hybrids of Oenothera lata × O. gigas, the former with 14 and the latter with 28 somatic chromosomes, show in the diakinesis and metaphases of the first reduction division, 21 chromosomes which give little or no evidence of pairing. In the heterotypic division these 21 chromosomes are distributed as nearly equally as possible between the daughter nuclei, one getting eleven and the other getting ten chromosomes.

Strasburger (100), commenting on Gates' observations, is of the opinion that the single equatorial plate stage which Gates has figured and which Strasburger has reproduced in his plate might easily be conceived to show seven single and the seven pairs of chromosomes,—with a single member of a pair lacking, which, according to the theory of the pairing or homologous chromosomes, should be present. Still, such pairing as is shown in this figure does not go far, to say the least, in establishing any theory of the pairing of homologous chromosomes.

There seems thus to be considerable evidence that there is a pairing and conjugation, more or less intimate, of the paternal and maternal chromosomes, occurring during or shortly before the prophases of the first reduction division. But in regard to the question as to how the chromosomes are paired there is a marked difference of opinion. Allen, Gregoire, Strasburger and others have interpreted the phenomena as showing pairing of chromosomes side by side, while Farmer and Moore, Mottier and others believe the pairing of the parental chromosomes to be end to end.

The voluminous literature of this controversy has been reviewed recently more or less fully by Strasburger (97, 98, 99), Overton (74), Mottier (60), Gregoire (31) and many others, and it seems superfluous here to give a detailed review of the subject. Strasburger (99) calls attention to the fact that each new investigation on the subject of reduction has only intensified the controversy instead of bringing the opposing views nearer to a general point of agreement.

Yamanouchi (111) reports that in Fucus there is an end to end pairing, while in Nephrodium (110) he calls attention to a side by side pairing. He seems to be of the opinion that these differences are to be found throughout the plant kingdom.

Juel, who in 1905 (46) reported the side by side pairing of spirem threads in the heterotypic prophases of Hieracium, later reports in Saxifraga (47) the looping of the spirem and probable approximation of the limbs of the loops to form the heterotypic chromosomes.

Gates (30) referring to Yamanouchi's results says "It is very evident that the time has passed when all accounts of reduction in plants can be brought under a single scheme." Botanists have not accepted this view to any extent as yet, and in view of the remarkable uniformity in the phases of typical cell division in the higher plants and animals, such wide difference in the reduction divisions, which themselves show a remarkable uniformity in general appearance wherever they occur, seems very improbable.

Almost all recent researches on the reduction divisions have emphasized the fact of the universal occurrence of the synaptic stage. Guignard however in 1899 (35) reported that he was unable to find the synaptic contraction in Naias major and therefore he concluded that synapsis was an artefact. In two other cases synapsis has been reported as lacking,—the case of the spermatogenesis in Triton, according to Moore and Embleton (62) and that of the ovogenesis of Planaria as reported by Schleip (87).

On the other hand Miss Sargent (83), Overton (73), Berghs (5), and Schleip (88) have reported synapsis as distinguishable in living cells. Schleip figures this stage from living material.

Schaffner (84, 85, 86) is of the opinion that the synaptic knot is an artefact due to fixation. In his recent work on Agave (86) he reports that in living pollen mother cells no trace of synaptic contraction is to be found.

McClnug's (51, 52) work on the insects has also led him to the conclusion that the synaptic contraction is an artefact.

Mottier (65) has regarded the synaptic knot as due to fixation and in a recent paper (67) has offered evidence to that effect. He has fixed embryo-sac mother cells of Lilium, some with the nucellar tissue intact and others with the tissue cut away so as to bring the mother cell close to the surface. In the untrimmed ovules he figures formless masses at the time of synapsis and in the trimmed ovules a fine spirem may be seen. His results thus suggest that the dense, contracted masses are due to fixation. It would seem that embryo-sac mother cells were not favorable for such a comparative study as this because of their isolation one from the other. In the Lily anther several mother cells may easily be brought into direct contact with the fixative by cutting, while those deeper will be less exposed. These large anthers have been more frequently studied than those of any other genus, and all accounts agree that synapsis is here entirely independent of proximity to cut surfaces in fixation.

Janssens (44) believes that in the spermatogenesis of Batrachoseps the characteristic synaptic figure is found only in those cases in which the fixation is poor. He finds that commonly the peripheral spermatogonial nuclei of a testis show no well defined synaptic contraction while in the deeper lying nuclei contraction is conspicuous.

Berghs (6) is inclined to believe that the synaptic contraction occurs normally though it is accentuated by the action of the fixing agent, and in this respect Lerat (49) is practically in accordance with him.

Häcker (38) who formerly (37) regarded the synaptic knot as natural is now "mehr als früher geneigt, auch den synaptischen Knaüel für einen nicht ganz natürlich Zustand zu halten."

By far the greater proportion of cytologists however are of the opinion that the synaptic contraction is a normal stage in the heterotypic prophases.

As to the mechanics of the synaptic contraction there is very little definite evidence. The shape of the knot as well as its location in reference to the nuclear membrane seem to indicate that it is due to some internal contraction and not simply a collapse of the chromatic nuclear content. Gravity, necessarily the important factor in producing a collapse, very evidently has nothing to do with the form and position of the synaptic knot, although Cardiff (14) has offered this explanation of the position of the knot.

Rabl's (78) conception of the nuclear organization, involving as it does the permanency of the chromosomes and the permanent connection of the chromosome with a center, should apply equally well to the movements of the spirem during synapsis as during the later phases of the mitosis. Evidences of such permanent connection between spirem and the exterior are not lacking.

In the ascus it has been shown by Harper (39) and Miss Sands (79) that there is a permanent connection of the spirem with the centrosome. Many investigators have figured fine fibers in attachment with the spirem during synapsis and in the following prophases. Among these are Farmer and Moore (23), Mottier (66) and Overton (73). Marquette's (53, 54) work on Isoetes and Marsilia has demonstrated that in plants lacking centrosomes there may still be a polar organization of the cell. It is possible that in those forms whose nuclei lack permanent centers there may still be a permanent connection of the chromatin with the cytoplasm.

There is thus much to suggest that the phenomenon of synapsis, as well as the recovery from synapsis and the later movements of the spirem or its segments, previous to the formation

of a conspicuous spindle, as well as after, may be due to the action of contractile fibers which in the earlier prophases owing to their delicacy are broken and shrivelled during fixation.

R. Hertwig (41), seeking to harmonize his "Kernplasmarelation" with the fact of the increase in the size of the animal egg without accompanying nuclear division, has proposed the idea that synapsis is an abortive cell division. The longitudinal splitting of the spirem he regards as a tentative bipartition which does not materialize, but from the standpoint of the multiplication of the chromatin has the same effect as a typical nuclear division. He offers as evidence that in Paludina and Periplaneta, as determined by his pupils Popoff (77) and Wassilieff (105) respectively, the chromatin seems to simulate a resting condition following the longitudinal splitting.

Hertwig's idea, aimed as it is to explain the enormous growth of animal eggs without nuclear division, does not apply at all to plants. Here the reduction division and ovogenesis may be separated by many cell generations, as is the case in the formation of the enormous egg of the Gymnosperms.

The second contraction figure or "second synapsis" seems to have been first described by Miss Sargent in 1896 and 1897 in Lilium Martagon (83). She describes this stage as "accompanied by all the signs of synapsis," appearing after a period of uniformly distributed spirem. Since then it has been reported by a number of investigators for a variety of species and has besides been plainly figured by several authors who have however failed to recognize or describe it.

The second contraction stage has been reported in the following plants: Lilium Martagon (Miss Sargent 83), Lilium candidum (Farmer and Moore 23, Mottier 65), Lilium Canadense (Allen 2), Lilium tigrinum (Schaffner 85), Lilium speciosum (Gregoire 31), Allium Moly (Miyake 55, Mottier 65), Hyacinthus orientalis (Hyde 42), Tradescantia virginica (Farmer & Shove 24), Podophyllum peltatum (Overton 73, Mottier 66), Oenothera rubrinervis (Gates 30), Oenothera

grandiflora (Davis 18), Primula sinensis (Gregory 32), Osmunda regalis (Farmer and Moore 23), Aneura pinguis (Farmer and Moore 23), and Humaria rutilans (Miss Fraser 29).

Farmer and Moore (23) have also reported the second contraction stage as occurring typically in the ovogenesis of Periplaneta americana, while Moore and Robinson (63) reported it in the spermatogenesis of the same species. Farther than this practically no attention has been called to this stage in the reduction divisions in animals.

The second contraction stage as described for the above species, with the exception of Oenothera, occurs after the stage of the uniformly distributed thick spirem. The characteristic feature of this stage consists in the massing of a considerable part of the spirem in the center of the nucleus from which mass more or less irregular loops extend to the periphery.

The case of Oenothera as reported by Davis (18) differs from the other cases above mentioned in that the second synapsis is described as following immediately after synapsis without the intervention of a stage in which the spirem becomes uniformly distributed.

Farmer and Moore are of the opinion that it is in second synapsis that the lateral approximation of the parental chromosomes which are to form the double heterotypic chromosome occurs. The parental chromosomes, having previously been connected end to end to form the continuous spirem, now become bent together in such a manner that the members of the pair come to lie side by side, forming thus the double heterotypic chromosome. The significance of the first synaptic aggregation is left undiscussed by Farmer and Moore, notwithstanding the fact that it is one of the most constant and typical stages of the reduction divisions. They describe "the first contraction stage" as occurring immediately after the formation of the thin spirem from the reticulum and the second contraction as following after a period of uniformly distributed thick spirem. Thus from their descriptions of these stages it seems clear that

no confusion exists in their minds as to the distinction between them.

It is to be noted that Farmer and Moore found the second contraction stage well marked in all the forms which they reported on,—in Lilium, in Osmunda, in Aneura, and in the ovogenesis of the cockroach. It may be of further significance in this connection that out of the 17 citations above of authors reporting the second contraction stage, 13 cases have been reported by investigators seeking to uphold the end to end pairing of parental chromosomes with the subsequent bending to form the heterotypic chromosomes.

Fraser and Brooks (29) have reported a "first contraction stage" and "a second contraction stage or synapsis" in the reduction divisions in the ascus of Humaria rutilans. Here the situation is complicated by the fact that according to Fraser and Brooks the nuclear fusion in the ascus is preceded by "the first contraction stage" in the nuclei which fuse, and immediately follows the recovery from this contraction. The fusion takes place while the two nuclei are in the split spirem stage, and the second contraction takes place therefore after the fusion of the two nuclei. Just how these two spirems are to become associated to form the loops of the second contraction stage the authors do not show.

That the second contraction figure has been observed by other investigators and has not been recognized by them seems clear from an examination of the figures of various authors representing the later prophases of the reduction divisions.

Strasburger (96) in his Figures 84 and 85 shows good second contraction figures for Tradescantia virginica. He calls attention to this massing in the center of the nucleus but makes no reference to the work of Miss Sargent and others on this stage. Ernst (22) represents a good second contraction figure (Fig. 7) for Paris quadrifolia which he describes as a late stage in synapsis, but from the thickness of the spirem strands it seems clear that the stage of the uniformly distributed spirem has been overlooked and that his figure really represents second synapsis. Berghs (7) (Fig. 3) shows a similar figure for the

same plant but makes no reference in his paper to the contracted condition. Juel (46) figures the stage for Hieracium (Fig. 52) and describes it as frequently following the homogeneous pachynema spirem, often forming a knot, "der jedoch mit der Synapsis wenig Aehnlichkeit hat." Cardiff (14) says that he could find "no constant and definite stage that could be called a second contraction period" in the plants which he studied. Yet in his Figures 47 and 48 for Salmonia biflora he shows, in what he describes as "post-synaptic nuclei," good representations of the central tangle of the spirem, characteristic of the second contraction period. His figure 69 for Botrychium obliquum, although designated as a stage in recovery from synapsis, suggests very strongly a later prophase and represents well the appearance of the second contraction figure.

In the heterotypic division in the formation of the germ cells in animals, a stage resembling the second contraction seems to be not uncommon.

Eisen (20) describes and figures in the spermatogenesis of Batrachoseps, "the angular spirem stage" which has the general appearance of the second contraction figure and occurs in the prophase following a period of extended thick spirem.

Janssens (43, 44) and Janssens and Dumez (45) describe the stage of "tension nucleaire" which they believe to be responsible for the transverse segmentation of the spirem in late prophases. In the spermatogenesis of the Tritons, of Batrachoseps attenuatus and Pletodon cinereus, this stage has been described as occurring in the late prophases after the longitudinal splitting has taken place. The loops formed at the time of the bouquet stage are described as extending away from the polar region by way of the periphery of the nucleus and returning by way of the interior. Attachment of the spirem to the nuclear membrane occurs at the polar region and in the regions where the loops extend along the periphery. When the spirem thread contracts it produces the stage of "tension nucleaire" which produces in the center of the nucleus "un noeud en partie inextricable" the nature of which in later stages becomes clear. Janssens believes that this stage of tension corresponds to the

"angular spirem stage" of Eisen. The tension on the nuclear membrane causes the nuclear cavity to grow smaller at this stage, but finally the spirem becomes broken apart at the weakest places which are the points of the connection of the chromosomes. Janssens is of the opinion that this stage is improperly called second synapsis.

Van Molle (57) in an investigation of the spermatogenesis of the squirrel divides synapsis into three stages, "lepto-synaptine," "amphi-synaptine" and "pachy-synaptine." His Figure 13, representing a thick split spirem massed in the center of the nucleus, is interpreted by him as the latter stage, but is in every way a good representation of the second contraction figure.

Arnold (3) in his Figures 12, 13, and 14 of the spermatogenesis of Hydrophilus piceus represents a stage which he interprets as post-synapsis. The chromatin, according to Arnold, is aggregated in the center of the nucleus in a dense knot, while from this loops radiate into the surrounding clear space in a manner characteristic of the second contraction. Arnold however calls these loops "linin" though they are figured as strands as thick as spirem strands.

Schleip (88) in the ovogenesis of Notodromas monarcha shows in his Figures 66 and 67 a stage following the uniformly distributed spirem after synapsis, in which the major part of the spirem is aggregated in the center of the nucleus with radiating loops of a split spirem. It is a typical second contraction figure and coincides with this stage as to time as well.

Gates (30), referring to the fact of the non-occurrence of the "bouquet stage" in the higher plants, is of the opinion that "the second contraction phase probably corresponds to it."

I have reviewed the literature pertaining to the so-called atpical methods of embryo-sac formation in an earlier paper (50). Since the appearance of this paper Miss Stephens' preliminary note (93) on the Penaeaceae has been followed by a more detailed account (94). She has found that the 16-nucleate embryo-sac of this group arises by four divisions of the embryo-sac mother cell,—her earlier observation that an axial

row of cells was present having been found to be incorrect. As stated in her preliminary note, however, she finds the 16 nuclei are formed in four definite peripheral groups due to the double division of the four peripherally placed nuclei resulting from the first two divisions of the mother cell. Three of each group form cell membranes about themselves; each of the four "peripheral groups" of cells so formed resembles an egg apparatus, while the remaining four nuclei fuse to form the endosperm nucleus. There is some variation in the number of nuclei entering into the fusion nucleus,—six cases being noted in which more than four nuclei fused. In these cases there seemed to be a corresponding diminution in the number of the nuclei to be found in the peripheral group of cells.

The embryo is usually formed by the fertilization of the nucleus of one of the cells of the "peripheral group" nearest the micropyle, at the apex of the embryo-sac, though cases are cited of embryo formation away from the apex. One case of polyembryony was noted with a rudimentary embryo at the apex of the embryo-sac cavity and another laterally placed.

The author is inclined to regard the first four nuclei formed by the division of the embryo-sac mother cell as macrospore nuclei, "the germination of each ceasing at the four-nucleate stage." She is further of the opinion that the embryo-sac of the Penaeaceae is to be regarded as a derived structure and not as primitive.

Went has supplemented his earlier paper (106) on the Podostemaceae by a fuller account (107). He describes the external morphology as well as the morphology of the embryo-sac of twelve species of the group representing the genera Oenone, Apinagia, Lophogyne, Mourera and Tristicha. His general conclusions as to the embryo-sac formation in the group coincide in detail with the results given in his preliminary paper. He finds uniformly throughout the species studied that the embryo-sac mother cell divides to form two cells, the outer of which degenerates. The nucleus of the inner cell divides, though neither cell wall nor cell plate is formed. The inner of the two nuclei, which Went calls the antipodal nucleus,

degenerates, after which the remaining single nucleus divides twice to form the four-celled embryo-sac, which consists of two synergids, the egg and the upper polar nucleus.

Went has figured and described synapsis as occurring in the mother cell of Oenone Imthurma previous to the first division of the embryo-sac mother cell, which seems to point clearly to the fact that the reduction divisions coincide with the first two divisions of the mother cell.

He also finds consistently in all forms studied the large pseudo-embryo-sac which is formed at the base of the small true embryo-sac and into which the embryo grows after fertilization, the true embryo-sac degenerating.

The more recent literature of polyembryony has been admirably summarized and the different methods of the formation of embryos classified by Ernst (21). Since Ernst's review Murbeck (89) has reported embryos arising from nucellar tissue in Alchemilla pastoralis and embryos arising from the synergids in Alchemilla sericata. Guignard (35) has also reported embryos arising from the synergids of Naias major. Treub (103) has reported the occurrence of the peculiar endosperm embryos in Balanophora elongata. Cook (17) has recently reported polyembryony in Mangifera indica and in Eugenia Jambos, the embryos arising from the nucellus.

The present study of Smilacina racemosa was originally undertaken with the aim of comparing the macrospore formation and embryo-sac development in this form with that in Smilacina stellata (50). The problem has since been extended to include the embryo development as well as the reduction phenomena in the pollen mother cells.

The materials for this study were collected in the vicinity of Beloit, Wis. during May and June of 1906, 1907, 1908 and 1909. Several fixatives were used but the material killed in Flemming's strong solution was finally used exclusively for the study of the reduction divisions while the weaker chrom-acetic fixatives gave better results with large embryo-sacs. Whole racemes were fixed and sectioned for the study of the pollen mother cells. For late stages of embryo-sac and embryo de-

velopment single ovaries were of necessity used. As far as possible the materials were fixed in the field, use being made of the simple air pump of the type described by Osterhout (72), to insure rapid penetration by the killing solutions. The sections were cut from five to 25 microns in thickness and stained in Flemming's triple stain and in Heidenhain's iron haematoxylin stain,—as well as in Benda's modification of the iron haematoxylin stain. The triple stain was used to the greatest advantage on thin sections and the haematoxylin stains gave best results on the thicker sections.

DESCRIPTIONS OF OBSERVATIONS.

1. The development of the embryo-sac.

The embryo-sac mother cell of Smilacina racemosa, at the time of synapsis, is commonly separated from the outer layer of cells of the nucellus by a single cell layer. In some ovules more than one cell intervenes between mother-cell and epidermis, though in these cases definite layers were not clearly distinguishable. Rarely the mother cell seems to lie in contact with the epidermis at the time of synapsis.

In a number of cases more than one mother cell was present in the same nucellus and the two were either in contact with one another or separated by a thin layer of somatic cells. Two cases were observed in which two fully formed embryo-sacs were lying side by side in the same nucellus. In one nucellus the embryo-sacs were lying in contact with one another and in the other they were separated by a thick layer of sterile cells. This latter nucellus was very broad and at the tip showed evidences of lobing.

The first division of the embryo-sac mother cell is in a plane approximately at right angles to the long axis of the nucellus (Fig. 36). The nuclear phenomena of this division indicate clearly that it is the heterotypic division. The short, thick bivalent chromosomes which appear in diakinesis, and the precocious splitting of the daughter chromosomes in the early ana-

phases of the first division both point conclusively to the heterotypic nature of this division. The number of the chromosomes was not accurately determined at this stage but it was unquestionably much smaller than the somatic number.

A cell plate is formed in the telophases of the first division which splits to form plasma membranes between which a rather thick, apparently gelatinous wall is deposited. Even in the metaphases the spindle is seen to be nearest the chalazal end of the mother cell and during cell plate formation the outer cell grows rapidly while the inner one grows very slowly, in some cases evidently not at all, so by the time the daughter nuclei are ready for the second division the outer cell is several times larger than the inner. Although considerable difference exists between the daughter nuclei it is not as striking as the difference in size between the two daughter cells.

The second or homoeotypic division occurs immediately after the formation of the daughter nuclei and cells of the first division. Usually the nucleus of the larger outer cell divides slightly in advance of the nucleus of the smaller inner cell (Fig. 37). It is possible that at times the inner daughter nucleus does not divide although I could find no positive evidence on that point. In the telophases of this division a definite cell plate is formed which splits and thus causes the complete separation of the four macrospores though no cellulose wall is formed (Fig. 38). These four macrospores are not uniform in size as are the four macrospores of S. stellata, the two outer cells being large and plump while the two inner ones are small and often distorted. Nevertheless the evidence is perfectly clear that for a time four, perfectly distinct cells exist. The lack of cell walls separating all four macrospores does not in the least affect their individuality as cells.

The plasma membranes which are formed by the homoeotypic central spindle figures do not form any cell wall as far as I have been able to observe and they very shortly disappear (Figs. 39 and 40). The plasma membrane and wall formed by the central spindle of the first division figure persists and we therefore get at this stage, two binucleated cells which have

resulted from the two divisions of the embryo-sac mother cell. The inner of these cells is small, with small, often distorted nuclei and the outer cell is at the close of the second division, commonly five or six times as large as the inner cell and its nuclei are plump and normal.

The outer binucleated cell grows rapidly and continuously to form the young embryo-sac. The inner cell however remains approximately the same size as at the time of the homoeotypic division and its nuclei become gradually distorted and disorganized, though often retaining their general outlines as late as the beginning of the development of the embryos. At that time they may be seen as small irregular masses of deeply staining material lying near the three antipodal nuclei which they greatly resemble.

When the outer cell has grown to two or three times the size it had at the time of the homoeotypic telophase, its two nuclei undergo division forming the four celled stage of the embryo-sac (Figs. 41 and 42). After a further period of growth during which the large central vacuole of the mature embryo-sac is finally formed, these four nuclei divide to form the eight nuclei of the embryo-sac (Figs. 43 and 44).

At a stage very shortly after this last division, three plump normal nuclei are to be seen in the micropylar end of the embryo-sac and three somewhat distorted nuclei in the constricted antipodal end, while the two polar nuclei have begun to approach one another (Figs. 45 and 46). At a somewhat later stage membranes are formed around the micropylar nuclei forming an irregular group of cells. There is a great diversity in the arrangement of the cells of these micropylar groups as well as in the size of the cells which compose them. Apparently they very rarely become organized to form the typical egg apparatus. I have seen, very rarely, embryo-sacs which had complexes in the outer end of the cell which might be interpreted as an egg apparatus. In nearly all the sacs examined the cells of the micropylar end were irregular in size, number and arrangement, bearing no resemblance whatever to an egg ap-

paratus and lacking any cell which might be regarded as an egg cell.

At about the time of the formation of the cell membranes about the nuclei in the outer end of the embryo-sac, the two polar nuclei have come together in the central part of the sac. They remain in contact, but entirely distinct from one another, until about the time of the beginning of embryo formation, when they are seen to be fused.

During the rapid growth of the embryo-sac the nucellus has been enlarged, to accommodate the growing cell, by a considerable increase in the size of the cells of the outer layer of the nucellar wall and by anticlinal division of some of these cells. Disintegration and absorption of the inner cell layers of the nucellus follow until at the upper end of the nucellus there remains but the outer layer of cells. In the immediate vicinity of the micropyle however, certain cells of the inner layers of the nucellus remain plump and dense and even increase in size. Ultimately, during the increase in the size of the embryo-sac and the disintegration of the neighboring cells these persistent cells become more or less isolated, round up and become approximately spherical. (Figs. 44, 45, and 46.) The number of such cells is variable and seems to range from one to four or five. Frequently small rounded cells which have earlier resisted absorption succumb and are absorbed thus diminishing the number of these cells.

As suggested above, the cells derived from the nucellus tend to become separated from the nucellar wall and thus come to lie within the outlines of the space occupied by the embryo-sac. In many cases such cells could still be distinguished from those of the embryo-sac (Figs. 47, 48 and 49) but in other cases, especially in the older stages it was impossible to distinguish between these rounded nucellar cells and those of the gametophyte. Although there was considerable variation in the size and content of the nuclei of the embryo-sac in this region, still no sharp distinction in appearance could be made between those of the sporophyte and the gametophyte.

In many preparations, in which the cytoplasm of the embryo-sac had shrunken away from the nucellar wall at the outer end a fairly accurate distinction could be made between the cells of the embryo-sac proper and those of the nucellus (Figs. 46 and 47). From these preparations it seems clear that part or all of the three micropylar embryo-sac nuclei undergo an early degeneration. Occasionally a larger cell persists in the cytoplasm of the embryo-sac which may possibly be the egg. Other preparations show all three cells degenerated which may indicate that a large cell persisting in the embryo-sac is simply one which has resisted degeneration longer than the other two (Figs. 48 and 49).

As above mentioned some of the rounded isolated nucellar cells at the micropylar end of the nucellar cavity become gradually absorbed. Others retain their plumpness and increase in size, some of them becoming considerably vacuolated. These proceed to form adventitious embryos. At a period before the fusion of the polar nuclei some of these rounded cells which are of undoubted nucellar origin, are seen to be dividing (Fig. 48). Apparently not all of these nuclei divide at such an early stage but certainly some divide before there is any possibility of fertilization having occurred. The nuclei of others of these nucellar cells seem to remain undivided often until after the fusion of the polar nuclei and the first division of the resulting endosperm nucleus.

Commonly these divisions take place in isolated cells, or at least in rounded cells which though lying near one another, seem to remain perfectly independent. The cell masses resulting from the division of these neighboring rounded cells seem to remain separate from one another, suggesting that each mass may ultimately form an embryo. Still in cases where some of a mass of cells were observed to be dividing it was of course impossible to determine whether the mass had resulted from the division of one or more than one cell. The frequent large solitary dividing nucellar cells suggest very strongly that in many instances at least, these adventitious embryos are derived from a single sporophytic cell.

The evidences are strong that in addition to this polyembryonic budding from the nucellus fertilization occurs. Pollen tubes are very abundant in the tubular styles and are not infrequently found in the vicinity of the micropyle and in several cases were seen entering the micropyle. The confused collection of nuclei of various sizes and stages of degeneration at the outer end of the nucellar cavity makes it very difficult to identify a male nucleus. The lack of any definite easily recognizable egg cell has also been a great obstacle in determining whether any nuclear fusion occurs here.

In order to determine whether pollination was necessary to the growth of embryos I castrated, in the spring of 1908, from 75 to 125 flowers on each of thirteen racemes, after which each raceme was carefully bagged to prevent pollination from other plants.

All of the flowers thus treated, probably from 1200 to 1400, withered and dropped off within three or four days. This shows conclusively that, although an embryo is probably rarely developed from the egg, still pollination is necessary to initiate the vegetative budding of the nucellar cells.

As will be seen from figures 50, 51, 53, 54, 55, considerable variation exists in the form and arrangement of the cells of these nucellar proliferations. Some have very broad bases, which suggests that they may have originated from more than one cell. Others have but one cell in contact with the nucellar wall (Fig. 52). In all these embryos the irregularity in the arrangement of the cells is conspicuous. It is also to be noted that no embryos are to be found which resemble embryos commonly figured as developing from an egg.

These adventitious embryos continue to grow and ultimately from one to three develop into mature embryos (Figs. 58 and 59). Usually all of these embryos appear perfectly normal and nearly equal in size. I have been unable thus far to germinate the seeds and so am unable to state whether all of the embryos will form plants.

I have been able, through the kindness of Prof. Osterhout who has furnished me with material of Smilacina sessifolia, to

compare the macrospore formation of this Pacific coast species with that of S. stellata. I find that the formation of four individual macrospores with the subsequent disappearance of the division walls occurs here identically as in S. stellata. This is, of course, to be expected since S. sessifolia is the Pacific form comparable to S. stellata.

Prof. H. D. Densmore has very kindly allowed me to examine his material of Smilacina amplexicaulis which is the Pacific Coast species very closely resembling S. racemosa. This species I have found to form four unequal macrospores in the same manner as S. racemosa. I have been unable to follow the development of the embryo-sac.

Preliminary studies on the early stages of the development of the embryo-sac of Maianthemum canadense show that here also separate macrospores are formed which later become merged into one cell. The four macrospores in this case seemed to be potentially equal as is the case in S. stellata.

It will thus be seen that Smilacina racemosa though differing definitely in the development of its embryo-sac from S. stellata as previously described by me (50) still resembles it in a striking manner. In S. racemosa as in S. stellata four distinct macrospores are formed which are separated from one another by distinct double membranes. These cells in S. racemosa are nevertheless not potentially equal for the two inner degenerate while the eight nucleate embryo-sac is formed from the products of the division of the two outer cells which first come to occupy the same cell by the disappearance of the plasma membranes between them. The absence of cell walls between the plasma membranes in no way detracts from the individuality of the four cells formed from the embryo-sac mother cell. If a cell wall were necessary to the individuality of a cell as Ernst (22a) intimates, separate individual cells would be extremely rare in the animal kingdom.

These results thus give additional evidence to support the view that the first four nuclei in the embryo-sac of the lilies and in other embryo-sacs which are formed directly from the embryo-sac mother cell, are morphologically macrospores. It is

plain that the embryo-sac of Smilacina racemosa is formed from two individual macrospores and the method of the formation of the macrospores here suggests strongly that all those embryo-sacs which are formed from one of the daughter cells formed by the first division of the emryo-sac mother cell are to be regarded morphologically as formed from two macrospores.

2. The development of the microspores.

In order to more accurately interpret the figures found in the reduction divisions I have first taken up a study of division in the somatic cells from which the spore mother cells are formed.

The resting nuclei of the cells just preceding the last premeiotic division are similar to those in the other embryonic areas of the sporophyte. The characteristic reticulum of small chromatin bodies connected by linin strands is clearly seen (Fig. 1).

With the initiation of the prophases of the last premeiotic division, the chromatin bodies lose some of their tendency to take the blue stain and take the red stain more readily. At this time they become arranged in more or less definite rows, connected by the linin elements, which take less stain than the chromatin bodies. In this characteristic manner the resting reticulum becomes transformed into a kinky, closely packed spirem. Whether there is ever a union of two or more net knots of the resting reticulum to form a single chromomere could not be definitely determined,—but from the irregularity in size of the chromatin masses of the reticulum and their comparative regularity in diameter in the spirem it seems very probable that some rearrangement is necessary to form the uniform chromomeres.

I was unable to find a paired condition of like chromosomes in the division figures of sporophytic nuclei such as Strasburger and others have seen in plant cells recently investigated by them. Their figures representing the pairing of somatic chromosomes are by no means convincing and it would seem that the foreshortening of those chromosomes seen from the end as well as the possible unequal rates of contraction of the chromosomes in the stages following the transverse segmentation of the

spirem, would introduce fertile sources of error in all attempts at determining the paired condition of sporophytic chromosomes.

The nucleoli of these resting nuclei vary as to size and number. Commonly three nucleoli of unequal size are present, but often only one is to be seen and in other cases as many as five or six smaller ones can be made out.

In the formation of the spindle of the last mitosis preceding synapsis broad strongly developed polar caps appear (Fig. 2). The spindle ultimately assumes the characteristic bipolar form of the typical mitosis.

In the metaphases and anaphases we find typical longitudinal splitting and separation of the chromosomes (Fig. 3). The chromosomes differ strikingly in their form, number and simplicity from those found in the corresponding phases of the heterotypic division. In the telophases the reconstruction of the resting reticulum of the daughter nuclei is brought about by the development of anastomoses between the daughter chromosomes. During this process the chromosomes retain for a time their boundaries even though they have become very much enlarged and reticulated (Fig. 4). Finally all outlines of the chromosomes are lost in the uniform reticulum and the nucleoli appear again in apparently the same number and sizes as before.

In none of the stages of this last division before reduction is there anything to distinguish it from a typical mitosis. There is nothing that can be interpreted as a preparation for the reduction divisions which are to follow.

The net knots of the resting reticulum immediately preceding synapsis are much more irregular in size and distribution than those found at the corresponding stage of the preceding cell generation (Figs. 5 and 6). Some of these knots are large and conspicuous and others are much smaller, appearing as fine granules connected by finer linin strands (Fig. 6). They are chromatic aggregations, irregular as to size and shape, connected by fine irregular strands which in staining take the same general color as the net knots. The strands connecting

these net knots do not differ essentially from the knots themselves in their staining qualities, though naturally, from their extreme fineness they are much fainter. I think that it would be difficult at this stage to demonstrate a difference between the two elements of the reticulum by their staining reactions. In later stages of mitosis this difference becomes more apparent.

Here as in the nuclei of the preceding cell generation there is no evidence of chromatic aggregations of the nature of prochromosomes, the net knots being too small and too irregular to be regarded as such, besides being far in excess of the sporophytic chromosome number.

The reticulum does not take up a marked peripheral position in the nuclear cavity as has been described for a large number of plants, though it is clearly less dense in the central region. There are also conspicuous clear zones around the larger nucleoli which are practically free from the reticulum.

The very first stages in synapsis are clearly shown in S. racemosa. The appearance of the first traces of a thin chromatic spirem and the first traces of the synaptic contraction are almost simultaneous as has been reported for various species by Mottier, Allen, and others. With low magnification this stage appears as though the reticulum had drawn together somewhat in certain regions, thus increasing the effect of knots by the apparent increase in the size of the chromatin aggregations (Fig. 7). At the same time areas are found at the periphery which are practically free from the reticulum. In the central region also areas are often formed which are nearly free from strands and knots.

High magnification shows in the nuclei at this stage, the presence of many, fine chromatin threads, much bent and folded on themselves in such a way that the appearance of knots is still present. Tangential sections of these stages show clearly that the apparent knots are due to such bendings and crossings and that the chromatin now forms a very fine spirem, which is bent and folded in a most tortuous manner (Figs. 7a and 8). Neighboring nuclei which with low magnification show little difference from the resting nuclei in appearance, show with

higher magnification the organization of the chromatin into threads with a disappearance of many of the fine linin strands.

As suggested above, the formation of the leptonema spirem results from a spreading of the chromatic material of the net knots along certain of the strands of the reticulum forming a thread into which is withdrawn others of these linin fibers. There is no definite arrangement of the knots into rows to form the spirem as seems to be the case in the formation of the spirem in the earlier mitoses of the anther. The knots of the reticulum placed side by side would, because of their great diversity in size, form a spirem of extreme irregularity and, in places, of much greater diameter than the leptonema spirem shown in the figure. It seems probable that some of the smaller knots lying side by side in the reticulum do form a part of a spirem, but nevertheless have no especial significance. Figure 9 shows a tangential section of a nucleus which lies in an anther with other nuclei in advanced stages of synapsis. Strands are still present but the appearance of chromatin knots is giving place to a condition in which the chromatin is distributed in threads. The disappearance of the fine strands of the reticulum together with the appearance of more open, free areas in the nuclear reticulum certainly suggests strongly the withdrawal of part of the fine strands into the knots or into the other strands.

We must conclude I think that the thin chromatin spirem is formed by two processes,—first the elongation of the net knots in certain definite directions along certain of the connecting fibers to form a much attenuated chromatic strand and secondly the withdrawal into the aggregation thus formed of the remaining linin strands.

Almost at the same time that the leptonema spirem becomes differentiated, the chromatic content of the nucleus can be seen to be irregularly drawn away from the nuclear membrane in places (Fig. 7). A tangential section (Fig. 7a) of the same nucleus as figured in figure 7 shows that the chromatin is probably all transformed into the spirem, though certain other areas such as are represented in figure 9 still show fine strands which indicate that a spirem of uniform thickness has not yet been

completely formed. The knotted appearance shown in this figure is due in part at least to the optical effect caused by a number of strands crossing in the same vertical plane,—the combined effect of these crossings being to allow less light to pass through these areas thus accentuating the effect of dark bodies or knots. This effect is of course increased by the fact of part of the spirem threads being more or less out of focus. The bending of a strand upon itself and the frequent folds and kinks all tend still further to give the nuclear content the appearance of a reticulum with conspicuous net knots.

It is not improbable that in some cases there has been a partial solution of the spirem threads by the fixing solution and a subsequent reprecipitation of the dissolved materials. Under such circumstances, whenever threads touched one another in crossing a knot would perhaps be formed.

Tangential sections show plainly the leptonema condition and in the median sections of the nuclei we can also now make out a tangle of more or less uniform strands rather than a mixture of very fine irregular anastomosing strands connecting unevenly distributed chromatic masses of extremely diverse sizes and shapes such as were present in the previous stage.

In the stage represented in Fig. 10 in which synaptic contraction is well begun leptonema strands paired for short distances are common. Slightly later stages still show this pairing for short distances. They also show diversity in the size of the spirem threads. Some are quite thin and others are of such size as could be expected from a fusion of the thinner strands (Fig. 12). These phenomena all suggest the gradual side by side pairing of parts of the thin spirem to form a coarse spirem such as appears in parts of Fig. 10. Here a part clearly consists of the thin spirem threads. A tangential section of the same nucleus shows the thin spirem with suggestions of pairing on the right side of the figure (Fig. 11). In other parts of the same nucleus the strands are distinctly larger and as in the upper central part of Fig. 10 paired strands can be distinctly seen extending for some distance. As contraction ad-

vances there is an increase in the proportion of the thicker threads in the nucleus.

The contraction of the thin spirem into the synaptic condition goes on rapidly after it is once inaugurated so that a single anther may often contain the earliest stages of contraction as well as nuclei with the chromatin practically at its maximum contraction into a tight knot.

The first perceptible stages of the synatic contraction, as has been already mentioned, consist in the drawing together of the kinky mass of fine spirem threads to form denser aggregations of the threads, at first principally at the periphery of the nucleus. This effect of peripheral aggregation seems to be produced by the collapsing of the chromatin threads at the periphery. The appearance of a withdrawal of material from the central region is thus given (Fig. 10). This peripheral collapse is quickly followed by a more or less uniform contraction leading to the formation of the globular synaptic aggregation at one side of the nuclear cavity, the familiar synaptic knot which is figured and described in all recent accounts of the reduction divisions (Fig. 13).

The synaptic knot of Smilacina is an especially dense globular mass lying in contact with the nuclear membrance. Very little detail can be made out in the knot itself, but thin tangential sections show it to be composed of a relatively thick spirem which in places can clearly be seen to be composed of two parallel strands. (Figs. 14 and 15). Occasionally strands of the spirem project from the synaptic mass and these can usually be seen to be double. A large proportion of the spirem of this stage, however, shows this doubling obscurely or not at all.

There is no orientation of the synaptic knot in any definite position in the nuclear cavity as far as I could determine. It is located neither in reference to gravity nor to the surrounding tapetal layer and the earlier stages of contraction suggest that the knot comes to lie on the side of the nucleus to which it is most closely attached by linin threads.

From the relative number of anthers in which the synaptic condition is to be found on a given raceme, it is very probable

that this stage occupies from ½ to ⅔ of the whole time consumed in the two reduction divisions,—from the first perceptible prophase of the first division to the telophase of the second. This observation accords in general with the observations of others in regard the duration of this stage.

The commonly described loosening of the spirem begins at once at the close of the period of synapsis. We first find loose loops extending out into the nuclear cavity (Fig. 16). Occasionally fine strands could be seen attached to these loops and connecting them with the nuclear membrane. Although the connections between spirem and the nuclear membrane are relatively infrequent in fixed material it is of course not impossible that in living material they are always present and that during the process of fixation they have been destroyed. These fine strands have been figured by Mottier, Berghs, Allen and other investigators for synapsis and the later prophases and they may also have some relation to the movements of the spirem during the prophases of this division.

As the spirem emerges from the synaptic condition it appears like a thick nodular filament, the nodules staining considerably darker than the main body of the filament (Fig. 16). The nodules are not arranged in two parallel rows on the periphery of the spirem but seem to be located on all sides of the filament. The bodies on the flanks of the spirem, as observed from above are more conspicuous, while those lying on the upper or under surface of the spirem are less easily identified. The double spirem of the synaptic stage must have fused to form this single spirem, at some time in the later phases of synapsis. This stage with its scattered chromatic granules may represent a stage in which the fusion is still incomplete for later the spirem becomes homogeneous.

After the spirem of the synaptic knot has begun to loosen it expands rapidly until it becomes uniformly distributed through the nucleus. (Figs. 17 and 18). The spirem is at this period a uniform homogeneous filament showing no differentiation into chromatin and linin nor into chromomeres. The nodular appearance apparent at first upon recovery from synapsis has dis-

appeared and the spirem is now a single thick thread. No definite arrangement of the spirem loops can be made out but the spirem is distributed in such a manner that no regions denser than others are present in the nucleus.

This stage has been described as occurring in the heterotypic prophases of practically all plants studied as to reduction. Still Gates (30) and Davis (18) have recently reported that in the Genus Oenothera the uniformly distributed spirem following synapsis is lacking, synapsis being followed at once by an irregularly distributed thick spirem. Davis is inclined to think that this expanded stage and the second contraction stage which follows it are both parts of the synaptic stage. This usage of the term synapsis seems to be new with Davis.

As the spirem begins to change from the uniformly distributed condition to a condition of unequal distribution it again appears double (Figs. 19 and 20). This separation appears first as a more transparent line containing less stain, which extends longitudinally through the middle of some parts of the spirem thread. The earlier stages are best demonstrated in preparations stained in Heidenhain's iron alum haematoxylin stain. Slides stained in Flemming's triple stain retain the stain more tenaciously between the halves of the spirem so that the longitudinal splitting shows more or less obscurely.

During the period of the uniformly distributed spirem the thread increases in diameter and at the same time probably shortens somewhat, though there is no conspicuous shortening. The increase in diameter is out of proportion to the shortening of the spirem and it seems clear that the amount of stainable material in the nucleus has been largely increased during this period.

This stage of the uniform distribution of the spirem does not seem to be of long duration for though not at all uncommon in my preparations it is quickly succeeded by a second contraction stage. A dense aggregation of a part of the spirem in the center of the nuclear cavity appears, with broad irregular loops or coils extending out from this central mass to the periphery (Figs. 21 and 22). This stage corresponds in appearance and

in time to the "second synapsis" first described by Miss Sargent for Lilium in 1896 and 1897, and since then reported by a number of investigators among whom are Farmer and Moore, Mottier, Schaffner, Gregoire, Allen and Miyake.

Figure 19 shows the beginning of this central aggregation drawn from a median section. Here it will be seen, the uniformly distributed condition has been converted into a system of irregular coils consisting of broad loops, no two of which lie in the same vertical or horizontal plane. It will be seen that a part of each coil extends to the periphery of the nucleus, where for a considerable portion of its length it lies near or in contact with the nuclear membrane. It is the crossing and recrossing of the inner parts of these coils in the central region of the nuclear cavity that gives the appearance of a central massing of the spirem which is characteristic of this period.

The spirem continues to shorten and thicken and as a result the inner portions of the coils are drawn nearer and nearer to the center of the nucleus thus increasing the density and compactness of the central tangle of threads. The peripheral parts of the coils do not change greatly in their configuration but remain open and for some distance in close proximity with the nuclear membrane, suggesting strongly that they are here attached to the membrane. (Fig. 21).

This attachment of one side of these coils to the periphery of the nucleus, the opposite side of the coil remaining unattached and free in the nuclear cavity further suggests a possible explanation of the central tangle of spirem strands,—for as the spirem shortens and thickens, as it clearly does at this period, the inner parts of the coils would be drawn toward the center of the nucleus and since they are being drawn in that direction from all sides they would necessarily cross and recross in such a way to produce a central tangle. This explanation of the phenomenon of "second synapsis" has been suggested by Janssens in his idea of a "tension nucleaire" which causes the segmentation of the spirem into chromosomes in Batrachoseps and in the Tritons. According to Janssens the loops of the bouquet stage following synapsis are whole chromosomes. They extend

out from the polar region to the periphery of the nucleus opposite. They are united with one another at the pole where they are also attached to the nuclear membrane. The other extremities of the loops are also in contact with and attached to the nuclear membrane. The loops being in this manner attached to the nuclear membrane at both extremities, when contraction of the spirem takes place, the state of "tension nucleaire" occurs and is relieved when the spirem breaks which, as Janssens believes takes place at the weakest place, the point of juncture of the chromosomes.

While a certain tension may exist in these nuclei the lack of polar organization makes a close comparison between them and Janssen's figures out of the question. Further the method of transverse segmentation here cannot differ greatly from such segmentation in typical mitoses where no such machinery seems necessary to the formation of chromosomes.

As the spirem gets shorter and thicker and the central tangle at the same time becomes more dense, the longitudinal segmentation of the spirem becomes more and more distinct, until at the time of the maximum density of the central knot the spirem is clearly split throughout its entire length so that in many places the halves diverge from one another for short distances. It is at this period that the spirem segments transversely in the peripheral region to form the heterotypic chromosomes. I did not find any instances in which the transverse segmentation took place elsewhere than at or near the periphery. In no case was I able to find free ends of the spirem in the central region of uncut nuclei.

Immediately following transverse segmentation the longitudinal halves of the spirem are seen to be twisted around one another forming the familiar strepsinema condition (Fig. 21). Previous to segmentation the twisting seems to be obscure or lacking. It is probably due to unequal shortening of the outer and inner parts of the halves of the split spirem. When the spirem is continuous or nearly so there is little chance for twisting to take place but transverse segmentation makes it possible.

The spirem segments formed by the transverse segmentation continue to shorten and thicken (Fig. 23). The central knot becomes looser and finally the chromosomes become separated out of the mass and we have the diakinesis stage (Figs. 24 and 25).

It will be seen from the preceding description and figures that there is nothing in the prophases of the heterotypic division of the pollen mother cells of Smilacina racemosa which suggests the approximation of the two limbs of a spirem loop to form a double heterotypic chromosome. Such a process could not possibly be fitted into the series shown in figures 17 to 25. Neither is there at any stage a sudden doubling of the thickness of the spirem, as would necessarily be the case if there were an approximation of the two limbs of a loop to form a heterotypic chromosome. The increase in the thickness of the spirem before and after transverse segmentation is gradual throughout the prophases. All possibility of the end to end pairing of two somatic chromosomes with a subsequent bending upon themselves and approximation to form the double heterotypic chromosomes is thus excluded in the heterotypic prophases of Smilacina racemosa.

Strasburger (98) has held that this second contraction phase is brought about by a grouping of the individual loops around the nucleolus. He suggests that some nutritive relation may exist by which the nucleolar material is added to the spirem. He believes that the presence of more than one nucleolus will cause a corresponding number of centers about which the spirem will mass or there may not be any massing at all.

Fig. 19 show that the central massing of the spirem may occur with nucleoli outside of the contraction figure. This is generally true in S. racemosa in which, as above noted, several nucleoli may be present. Although nucleoli may at times be included in the central mass it is clear that no relation here exists between the number and position of the nucleoli and the location of the central contracted spirem tangle. In no case did I find any appearance that could be interpreted as showing sev-

eral centers of aggregation of the spirem as Strasburger believes should be the case in nuclei with several nucleoli.

The coils of the second contraction figure extend toward the periphery of the nucleus in all directions making it very difficult to determine whether any relation exists between the number of coils and the number of chromosomes. If the transverse segmentation of the spirem takes place in the peripheral region of the nucleus the number of the chromosomes will equal the number of coils of the spirem. In the earlier diakinesis stages the presence of very short double chromosomes in the same nucleus with others of the same diameter but five or six times as long (Fig. 24) suggests the possibility of a later segmentation of some of the first formed spirem segments, for if the unequal lengths were due to a more rapid contraction of certain chromosomes we should naturally expect the shorter chromosomes to have a greater diameter. There is however a decided decrease in the volume of the individual chromosomes following their formation by the breaking up of the second contraction figure so that the chromosomes of the equatorial plate and metaphase are considerably smaller than those of early diakinesis. It is probable that this irregularity of the chromosomes of early diakinesis may be due to a more rapid decrease in volume as well as length in some chromosomes than in others. Polar views of the equatorial plate stage although showing a certain diversity in the size of the chromosomes do not show any such great difference as is seen in earlier diakinesis (Figs. 29 and 30).

There is however a difference in the size of the chromosomes as they appear in the equatorial plate stage. In polar views of the equatorial plate from five to seven chromosomes lying in the interior of the chromosome group are seen to be decidedly smaller than those lying around the periphery. There is also some irregularity in size among these peripheral chromosomes, one or more being usually larger than the rest. (Figs. 29 and 30). Figure 30 shows 24 double chromosomes and figure 29 shows 22. The former number is evidently the reduced number.

The multipolar spindle is formed from a felted zone as has

been frequently described and upon the breaking down of the nuclear membrane the double chromosomes seem to be pulled into the center of the nuclear cavity where for a short time they form a crowded mass in the center of the multipolar polyarch spindle. The chromosomes are then drawn out of the crowded central aggregation and form the equatorial plate. They at first become very much drawn out at the points of attachment of the spindle fibers as though considerable tension existed. This distortion disappears as the spindle figure becomes diarch and at the time of the equatorial plate stage no traces of it can be seen (Figs. 26 and 27).

As the chromosomes become arranged in the equatorial plate they have become shortened and thickened until their length is only about one and one-half times their diameter. In some chromosomes there seemed to be little or no difference in the two dimensions, the direction of the longitudinal segmentation being the only index as to which was the longer axis (Fig. 28).

There can be no question that the halves of the double chromosomes of the equatorial plate stage are the longitudinal halves of the spirem segments which were formed by the transverse segmentation of the spirem during the second contraction stage. The line of separation between the two halves of the heterotypic chromosome of the equatorial plate stage is the line of the first longitudinal segmentation of the spirem. This first longitudinal splitting which appeared before the second contraction stage, has remained distinct throughout all the phases of second synapsis and diakinesis. At no time has there been any closing up of the first longitudinal split and a folding of parts of the spirem upon one another to form the double heterotypic chromosome.

The halves of the double chromosomes in the equatorial plate are drawn apart in the manner so frequently described, the ends toward the center of the plate separating first while the peripheral ends are last to separate. The smaller chromosomes of the interior of the plate usually are some distance from one another before the larger peripheral chromosomes are completely separated. (Fig. 31).

As the halves of the double chromosomes of the metaphase are being drawn apart, the second longitudinal split appears. By the time the single chromosomes are fully separated from one another this longitudinal split is conspicuous, the resulting halves diverging widely from one another. They remain attached to one another at the end to which the spindle fibers are attached, giving the familiar V shaped appearance as they pass back to the poles (Fig. 36). The short, thick form is retained in the anaphase and not until the daughter chromosomes have reached the poles do they begin to elongate to form the slender V-shaped chromosomes characteristic of the earlier telophases. The apex of the Vs points toward the pole and the limbs radiate out from this as a center.

As the thick-V-shaped chromosomes reach the pole the limbs of the Vs become considerably extended and at the same time becomes more slender (Fig. 32). As they elongate they become constricted in places which gives them a knotty appearance (Fig. 33). It seems probable that part of these constrictions continue to grow deeper until the chromosome is almost separated in several places. By this time the chromosomes have lost their regular contour and have become so ragged and uneven that it is impossible to trace their individual outlines. At this stage one or more nucleoli become visible, indicating a resting condition of these daughter nuclei of the heterotypic division (Fig. 34). In this resting condition, however, it may be seen that there is no anastomosing of chromosomes to form a reticulum. The chromosomes seem to remain entirely free from one another although much constricted and extended in regions.

The chromosomes reappear in the prophases of the homoeotypic division in the characteristic V-shape of the early telophases of the heterotypic division (Fig. 35). They seem to be formed directly by a drawing together and smoothing up of their outlines. I could find no traces of anything that resembled a spirem stage following the resting condition. It is impossible to determine by direct observation in Smilacina that these chromosomes of the homoeotypic prophases are identical

with those of the heterotypic telophases. From the fact that large masses remain intact through the resting condition,—that the homoeotypic chromosomes are formed very quickly without the intervention of a spirem stage and from their general shape, size and position it seems reasonable to conclude that they are the same.

As these homoeotypic chromosomes become aggregated in the equatorial plate they have the general elongated form of chromosomes of typical mitoses and in the metaphases the two halves are separated one part going to each pole.

DISCUSSION.

The observations described above for Smilacina racemosa point strongly to the conclusion that in the early prophases of the first reduction division a longitudinal pairing of leptonema spirems occurs. The thin pre-synaptic chromatin strands frequently appear paired. The strands increase in thickness as the nucleus is entering into synapsis as would be the case if there were a lateral approximation of two thin spirems. Tangential sections of the synaptic knot frequently show that the spirem is here double. The simplest interpretation of these facts is that there has been a side by side pairing of thin chromatin strands during these early prophases.

These evidences are strongly supported by other phenomena of the later prophases. It seems clear: (1) that the longitudinal split of the later prophases does not close up but persists until the halves are finally separated: (2) that the transverse segmentation is not exclusively central, but in most cases at least, occurs near the periphery; (3) that the shortening and thickening of the spirem segments after the transverse segmentation, is gradual and not the sudden shortening and thickening that would necessarily result from the lateral approximation of the two limbs of a loop composed of two chromosomes attached end to end. As far as I am aware no investigator of the reduction divisions has called attention to any such sudden shortening and

thickening as should occur at this period on the theory of Farmer and Moore.

Mottier (67) though a supporter of the Farmer and Moore hypothesis is of the opinion that the segmentation of the spirem may take place at the periphery as well as in the central region of the nucleus. The central segmentation of the spirem appears however to be an essential part of the Farmer and Moore hypothesis. Very serious mechanical obstacles interfere with the looping and lateral approximation when segmentation occurs away from the central region. The looping of the spirem in the peripheral regions of the nucleus which is open and relatively free from spirem strands at this period seems to be the only method and the only position in which looping could be brought about. In the central region the presence of the aggregation of spirem strands prohibits looping until after the segmentation of the spirem and the breaking up of the central aggregation. It is difficult to conceive of the looping of spirem segments after segmentation and such a process has thus far not been described.

There can be no doubt that in Smilacina there exists a definite well marked second contraction period as a stage in the first reduction division. The survey of the literature indicates further that without doubt the phenomenon is more widespread than the attention which it has received would indicate.

The view of Davis (18) that the second contraction stage is simply a continuation of synapsis does not correspond well with the observed facts in Smilacina. The stage of the uniformly expanded spirem found here has been described for the heterotypic division in practically all the higher plants studied and has nothing in common with synapsis according to the commonly accepted usage of the term. The shortening and thickening of the spirem may be a more or less uniform, continuous process from the time of its formation to diakinesis but to designate this whole period "synapsis" because contraction is going on is to give an entirely new meaning to the term. There are two distinct stages in which the spirem becomes wholly (synapsis) or partly (second synapsis) aggregated in a dense knot. It is

this condition of aggregation which is the visible evidence of synapsis and we can not at present give it any more definite characterization. Between these two stages the spirem becomes expanded uniformly throughout the nuclear cavity and is perhaps here more uniformly distributed than in any other stage of its existence. As contrasted with the compact massing of the whole spirem in synapsis, the second contraction which follows the expanded stage of the spirem is only a partial aggregation of the spirem in the center of the nucleus with loops extending into the open regions of the nuclear cavity.

There is no evidence in Smilacina that the second contraction stage has any such significance as has been ascribed to it by Farmer and Moore. It is not a "true synapsis" according to the usage of Farmer and Moore since there is no lateral approximation of chromosomes which have been arranged in pairs end to end in the spirem.

Evidences of a connection of the nuclear contents with an external cytoplasmic center are common in animal cells and in the lower plants. The work of A. and K. E. Schreiner (89, 90) on Myxine and Tomopteris and that of Harper (39) on the mildews may be cited as typical cases. The work of Marquette (53,54) on Marsilia and Isoetes further suggests that in forms lacking a centrosome there may still be a polar organization and the possibility exists that in those forms lacking centers and without any visible polar organization there may still be a connection of the nuclear contents with the cytoplasm.

In Smilacina there are evidences of such a connection in the form of fine fibers extending from the spirem to the periphery of the nucleus. It must be said however that these connections are not as conspicuous as those which have been figured for various other species by Allen (1), Farmer and Moore (23), Mottier (66), Overton (74) and others.

The orientation of the spirem during the second contraction stage suggests strongly that certain regions of the spirem are attached to the periphery of the nucleus while parts unattached tend to be drawn into the center giving the familiar central aggregation characteristic of the phase. On this assumption the

second contraction figure would necessarily follow the continued shortening and thickening of the continuous spirem. It is my opinion that the second contraction figure arises as a necessary stage in the shortening of the elongated post-synaptic spirem to form the short heterotypic chromosomes.

That part of Janssen's (43, 44) hypothesis of a "tension nucleaire" which assumes the spirem to be attached in places to the nuclear membrane may possibly be applicable to the second contraction stage in Smilacina. Still that any tension exists in the nuclei of Smilacina at this stage seems improbable. The curvature of the coils and loops of the spirem is too great to be consistent with any great tension. Further this hypothesis as suggesting a method of segmentation seems to be superfluous for in ordinary mitoses no such mechanism is necessary for the segmentation of the spirem.

Overton's (7) suggestion that in plants with short chromosomes the second contraction stage is inconspicuous or lacking probably should be limited to refer to those forms whose nuclei have a relatively small amount of chromatin as is the case in Calycanthus and Thalictrum.

Strasburger's explanation (98) that the second contraction figure is formed around the nucleolus for the possible purpose of obtaining nutriment from it, fails with Smilacina for here several nucleoli are present and but one center of aggregation. The nucleoli are probably more frequently to be found outside of the contraction figure than included within it. The suggestion of Strasburger that in nuclei with several nucleoli several centers of aggregation or none could be expected, finds no substantiation in Smilacina for I have never observed more than one center of aggregation and have never observed nuclei of this prophase in which it was lacking. The second contraction figure is here quite unrelated to the position of the nucleoli.

Gates' (30) conception that the second contraction stage corresponds to the "bouquet stage" of Eisen seems based on the approximate time of its occurrence. In the "bouquet stage" the chromosomes become definitely oriented in respect to the centrosome. The stage is commonly described as occurring im-

mediately upon recovery from synapsis and is followed by a period of a more or less distributed condition of the chromatin. Janssens' stage of nuclear tension which may be comparable with the second contraction stage follows a definite bouquet stage. I have cited other cases [Eisen (20), Van Molle (57) and Schleip (88)] of a definite contraction stage occurring in the reduction divisions of animals which has been preceded by a bouquet stage and a period comparable to the stage of uniformly distributed spirem. It seems clear therefore that no relation can exist between the bouquet stage and the second contraction figure.

The work of Rückert (87) and Häcker (36) on Cyclops has shown that the parental chromosomes remain separate for a time at least in the nuclei of the diploid individual. The work of Moenkhaus (36) on hybrid fishes further suggests that there is not a fusion of the parental chromosomes at the time of fertilization but a gradual mingling of the distinct chromosomes in the nuclear cavity.

In Pinus Blackman (8), Chamberlain (15) and Miss Ferguson (26) as mentioned above, report that the parental chromosomes retain their identity during the first mitosis of the fertilized egg. The work of Woycicki (109) on Larix, of Murrill (70) on Tsuga, of Nichols (71) on Juniperus, as well as that of Shaw on Onoclea (91) and Dublin (19) on Pedicellina demonstrate similar phenomena.

The parental nuclei in the diploid generation of the rusts as shown by Blackman (9), Christman (6) and by others, remain perfectly distinct throughout the entire diploid generation. The fact that in a large number of cases among animals the sex nuclei at the time of fusion have their chromosomes already organized so that at least during the first cleavage of the fertilized egg the parental chromosomes must remain distinct bears out the general conclusion that the paternal and maternal chromsomes remain distinct during the first division, at least, of the diploid generation.

The pairing of the chromosomes as described for Funkia, Galtonia, Yucca, etc. shows that there is a tendency toward a

definite arrangement of the parental chromosomes and it seems probable that at some period during the diploid generation there is a distribution of homologous chromosomes to form pairs.

It is at the time of the formation of the spores at the close of the diploid generation that the chromosomes seem to come into close and intimate contact but no satisfactory evidence is yet available as to when or how the homologous chromosomes from the two parents have come into such relations that the synaptic pairing becomes possible.

The last typical divisions preceding synapsis in Smilacina, as described above, differ in no way from ordinary somatic divisions. There is no evidence that any preparation for reduction occurs immediately preceding the heterotypic division but it seems clear that there is an abrupt change from an ordinary division immediately preceding synapsis to the heterotypic division immediately following it. If there has been a pairing of homologous paternal and maternal chromosomes previous to synapsis it must have taken place at a period previous to the nuclear divisions in the young anther.

The conduct of the nuclei in the diploid generation of the rusts, which do not fuse until immediately before reduction indicates that here the pairing of the homologous parental chromosomes must take place after the last pre-reductional division, immediately after fusion and immediately preceding reduction.

The work of Rückert, Häcker, Moenkhaus as well as the above mentioned authors on the Gymnosperms indicates that the association of homologous parental chromosomes does not in the forms studied take place immediately upon the fusion of the gametes but that the chromosomes of the two parents may remain distinct for several cell generations, though finally becoming merged into one homogeneous group. Still the presence of paired chromosomes in somatic nuclei as reported in Funkia, Galtonia, Yucca and other plants suggests that there may be a relatively early pairing of homologous chromosomes. These phenomena may indicate that though the parental

chromosomes do not become immediately associated in pairs after fusion, in these forms, they gradually come to be so paired.

These facts suggest a possible explanation of the appearance of blend hybrids and of hybrids which segregate according to Mendelian ratios. The blends may occur in those cases in which the parental chromosomes become associated early in the diploid generation and thus exert a mutual influence upon one another. The hybrids which segregate in the second generation may be those whose chromosomes do not become associated until synapsis. The association of the parental chromosomes is here so intimate that the mutual influence which they exert upon one another is far greater than that which results from the less intimate association of the chromosomes in the somatic nuclei.

SUMMARY.

1. Evidence found in the pollen mother cells of Smilacina racemosa indicates that no preparation for reduction takes place in the last division preceding synapsis but that the last presynaptic division is an ordinary somatic cell division.

2. The observed phenomena during synapsis suggest very strongly that a side by side pairing and fusion of leptonema spirems occurs, and that the fusion is complete at the time of the recovery from synapsis.

3. The second contraction stage which follows a period of uniformly distributed spirem results necessarily from the gradual contraction of the spirem which is attached to the nuclear membrane in places.

4. There is no approximation of the limbs of loops to form the double heterotypic chromosomes.

5. The double heterotypic chromosomes are formed by the transverse segmentation of a longitudinally split spirem, the line of the split of which probably represents the line of approximation of the two parental spirems at the time of synapsis.

6. The first division of the embryo-sac mother cell of S. racemosa results in two unequal cells, the inner being much the

smaller. The homoeotypic division produces four unequal cells, which are fully separated by plasma membranes.

7. The plasma membrane of the first division persists while those of the second division quickly disappear, thus producing two unequal binucleate cells.

8. The inner of these binucleate cells does not undergo any further growth while the outer grows rapidly and by two nuclear divisions forms the eight nucleated embryo-sac.

9. Certain nucellar cells in the micropylar region resist disintegration and remain large and plump in the embryo-sac cavity and later divide to form adventitious embryos of which from one to four mature.

10. The egg apparatus of the embryo-sac is never well developed and it is practically impossible to identify the egg and to determine whether it also develops into an embryo.

11. The presence of abundant pollen tubes indicates that fertilization occurs.

12. Castrated flowers withered within two or three days showing pollination to be necessary to the development of the nucellar embryos.

In conclusion I wish to express my gratitude to Prof. R. A. Harper for his most helpful suggestions and criticisms during the preparation of this paper.

Since this paper went to press Lawson (Trans. Roy. Soc. Edinb. 47: 661.1911.) has advanced the view based upon work on Smilacina, that synapsis is not due to a marked contraction of the nuclear contents but to a sudden enlargement of the nuclear cavity which gives the appearance of a contraction. Gates (Ann. Bot. 25:909.1911.) believes that his observations on Oenothera gigas afford evidence in favor of this view.

I have made camera drawings of a large number of nuclei of Smilacina racemosa in the synaptic condition and preceding it and find no evidence whatever of an enlargement of the nuclear cavity at this period. Synapsis in this species is in every case observed a contraction.

The great number of figures of this phase published before Lawson's hypothesis furnish unbiased evidence that synapsis is a contraction of the nuclear contents.

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DESCRIPTION OF FIGURES

All figures were drawn with the aid of a camera lucida. Figures 1 to 35 inclusive have an approximate magnification of 2000 times; figures 36 to 46, 600 times; and figures 47 to 58, 150 times.

Plate I.

Figure 1. Resting nucleus immediately preceding the last pre-synaptic division in the anther.

Figure 2. Late prophase of the last pre-synaptic division Polar caps formed as in typical divisions.

Figure 3. Equatorial plate stage of the last pre-synaptic division.

Figure 4. An early telophase stage immediately preceding synapsis. The general location of the chromosomes can still be seen.

Figure 5. The resting nucleus of the pollen mother cell.

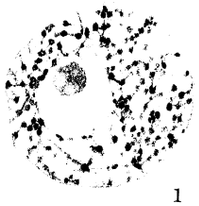
Figure 6. A tangential section of the resting nucleus of a pollen mother cell showing chromatin knots and the finer connecting strands.

Figure 7. The nucleus of a pollen mother cell in the early prophase stage. The net like appearance is becoming transformed into more uniform and continuous threads while the chromatin material is drawing away from the periphery of the nucleus.

Figure 7a. A tangential section of the nucleus shown in Fig. 7. The fine spirem is seen here to be well formed with no trace of knots and connecting strands.

Figure 8. Tangential section of another nucleus in the same stage of contraction as Fig. 7. The leptoneme condition conspicuous.

Figure 9. A tangential section of a nucleus in very early prophase. There are fewer connecting strands and the chromatin knots are more elongated than in the resting nucleus.



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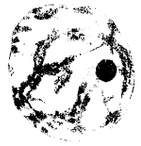

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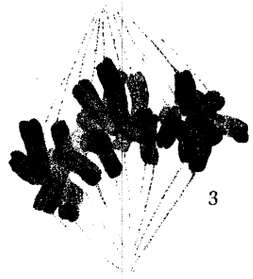

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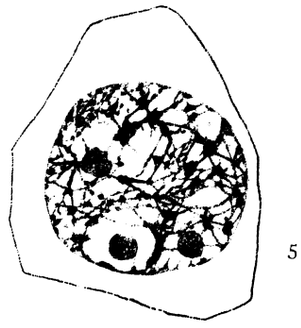

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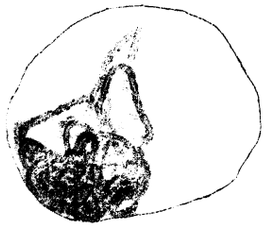

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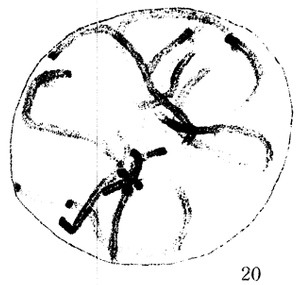

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Figure 10. Nucleus at a more advanced stage of contraction. Chromatin threads of two sizes can be seen here. Also definite double strands.

Figure 11. A tangential section of the nucleus represented in figure 10. Evidence is to be seen here of the approximation of two thin spirems to form one thick spirem.

Figure 12. A portion of a nucleus entering into synapsis showing chromatin threads of different diameters.

Figure 13. The fully contracted synaptic knot.

Figure 14. A tangential section from the synaptic knot in the above figure. The double nature of the spirem is clearly evident in a part of the strands.

Figure 15. A tangential section from another synaptic knot, showing doubleness.

Figure 16. A nucleus at the time of recovery from synapsis. The spirem shows some evidences of doubleness which may however be due to the rows of darker bodies along the periphery of the threads.

Figure 17. A post synaptic stage. The spirem is not quite uniformly expanded. The free ends showing are cut ends of the section.

Figure 18. A surface view of a nucleus in the uniformly expanded condition.

Figure 19. A median view of a nucleus in very early stages of the second contraction. The free ends to be seen here are ends of spirem thread which pass out of the plane of the section.

Figure 20. A view of a section of a nucleus entering into the second contraction, showing the distribution of the spirem threads at this time. The first longitudinal splitting is clearly evident here.

Figure 21. A nucleus in the second synapsis. The spirem is partly aggregated in the center only. Segmentation is taking place in the region of the periphery of the nucleus. This figure is from a nucleus uncut by the razor. Not all the spirem is drawn however.

Figure 22. A later stage in the second contraction. The nuclei in the anther from which this was drawn were unusually small. Some cut ends show in the figure.

Figure 23. The second contraction figure is becoming lost due to the separation of the chromosomes from one another due to shortening and thickening.

Figure 24. A later stage in the shortening and thickening of the spirem segments to form the double diakinetic chromosomes.

Figure 25. Early diakinesis. The chromosomes very irregular in size.

Plate II.

Figure 26. A cell in the multipolar spindle stage. Note the drawn condition of some of the chromosomes as though under tension.

Figure 27. A cell in which the multipolar spindle is becoming bipolar and the chromosomes are being drawn into the equatorial plate. Note the distorted appearance at the point of the attachment of the spindle threads.

Figure 28. The heterotypic metaphase stage.

Figure 29. A polar view of the equatorial plate stage. 22 double heterotypic chromosomes are present.

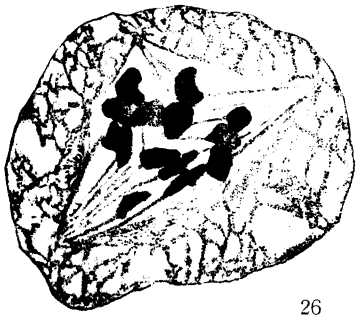
Figure 30. Polar view of heterotypic equatorial plate. 24 chromosomes.

Figure 31. Late anaphase heterotypic division. The V-shaped chromosomes arising from the second longitudinal splitting are to be seen.

Figure 32. A polar view of an anaphase somewhat earlier than that represented in figure 31: showing the V-shaped chromosomes.

Figure 33. A partial polar view of the early telophase of the heterotypic division. The chromosomes are becoming elongated and knotty.

Figure 34. A resting nucleus of the heterotypic division. Note the lack of a reticulum and the presence of a nucleolus.



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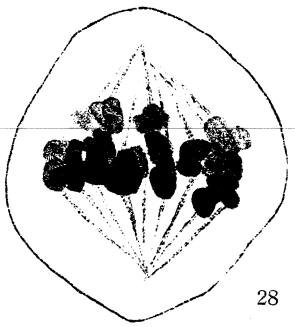

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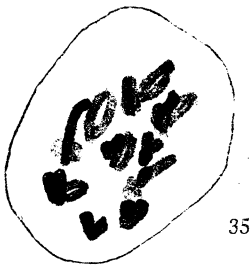

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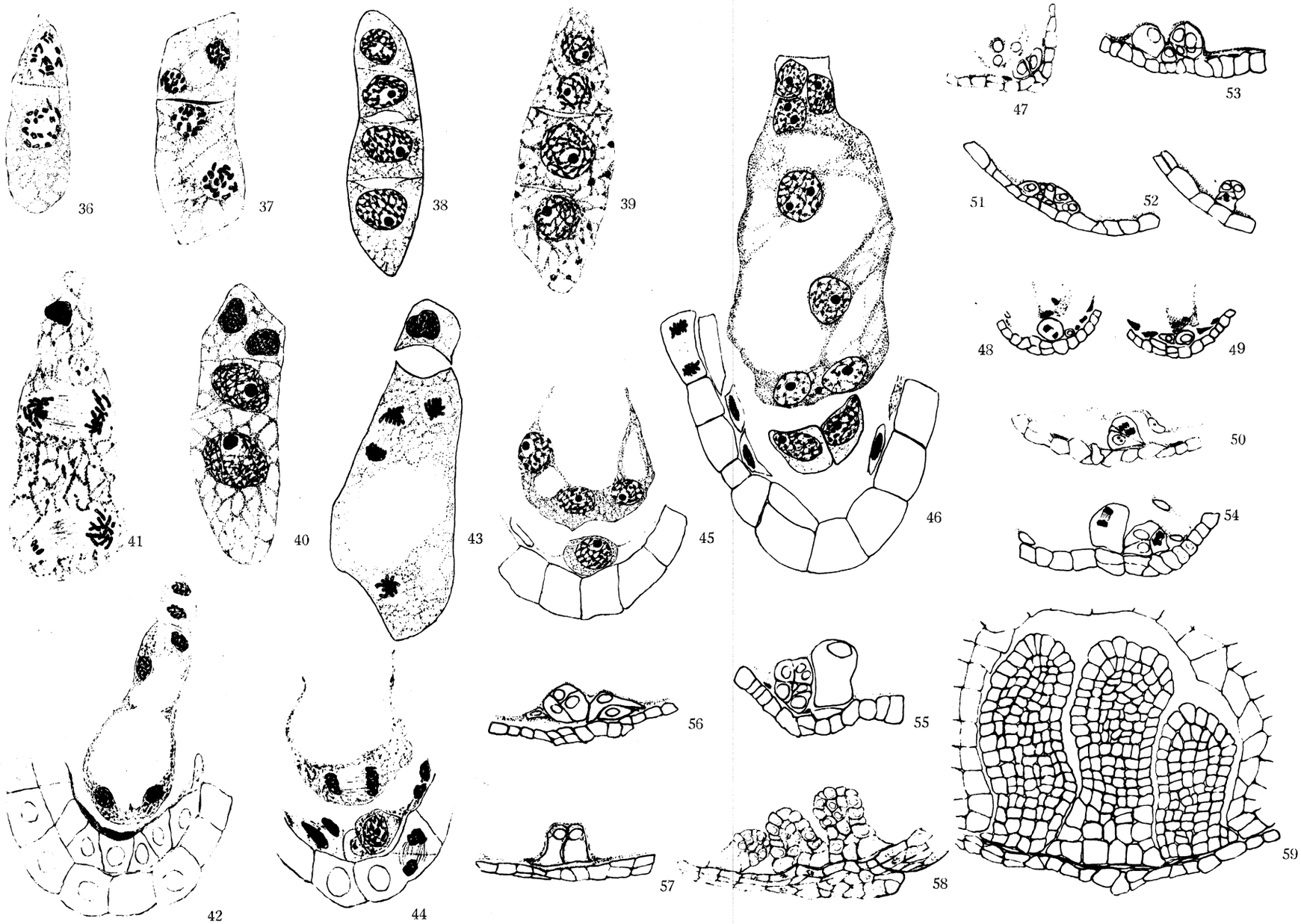

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Figure 35. A multipolar spindle stage of the second reduction division. The characteristic V-shape of the chromosomes of the late anaphases of the first division is again seen here.

Plate III.

Figure 36. A telophase of the first division of the embryo-sac mother cell. Note the dissimilarity of the two daughter cells in size.

Figure 37. Telophase of the homoeotypic division of the embryo-sac mother cell. A wall is formed between the daughter cells of the first division and a cell plate between the outer pair of nuclei.

Figure 38. Four fully separated macrospores formed from the two divisions of the embryo-sac mother cell. The inner spores are smaller.

Figure 39. A stage similar to figure 38, though later. No wall showing between the inner pair of nuclei while a trace of a plasma membrane shows between the outer pair.

Figure 40. The inner pair of nuclei small and evidently in early stages of degeneration. The outer pair large and healthy with no trace of division membranes between them.

Figure 41. Division of the two outer nuclei to form the four nucleate embryo-sac. The inner nuclei nearly degenerated.

Figure 42. The four nuclei fully formed in the embryo sac while the outer pair still retain their form in the small inner cell. The nucellar wall is here two cell layers in thickness.

Figure 43. The division of the four nuclei to form the eight nucleate embryo-sac. This embryo-sac was cut obliquely and in this figure the upper end is shown, and a polar view of one of the lower nuclei in process of division. The inner pair of macrospores is still to be identified.

Figure 44. The lower end of the same embryo-sac as shown in Fig. 43. The other nucleus of the sac is shown in the figure above. A large cell of the nucellar wall is seen immediately below the embryo-sac with remains of other cells which have degenerated. The nucellar wall has been reduced to a single layer of cells.

Figure 45. A view of the microplylar end of a nucellus showing three of the embryo-sac nuclei and a large nucellar cell of the inner layer which has resisted degeneration.

Figure 46. A fully formed embryo-sac with two cells of the nucellus separated from the nucellar wall and lying near the embryo-sac.

Figure 47. Nucellar cells persisting at the apex of the embryo-sac.

Figure 48. The embryo-sac nuclei degenerating while a nucellar cell is dividing.

Figure 49. The next section of the same nucellus showing another nucellar cell which seems to be healthy and normal. The endospern nuclei not yet fused.

Figure 50. An embryo formed from one of the dividing nucellar cells. Endosperm nucleus seen above it.

Figure 51. A flat aggregation of cells at the micropyle.

Figure 52. A section remote from the above represented Fig. 51 but in the same nucellar cavity. The embryo here is quite clearly from one cell.

Figure 53. Two rudimentary embryos lying close together in the micropylar region.

Figure 54. Two embryos in the micropylar region. One still a single cell in stages of division.

Figure 55. Two embryos much similar to those in Fig. 53.

Figures 56 and 57. Two successive sections in the micropylar region of a nucellus, showing three and probably four young embryos.

Figure 58. Three well-formed embryos in the micropylar region. The inner integument is shown.

Figure 59. Three large embryos in a seed in which endosperm is nearly all formed.